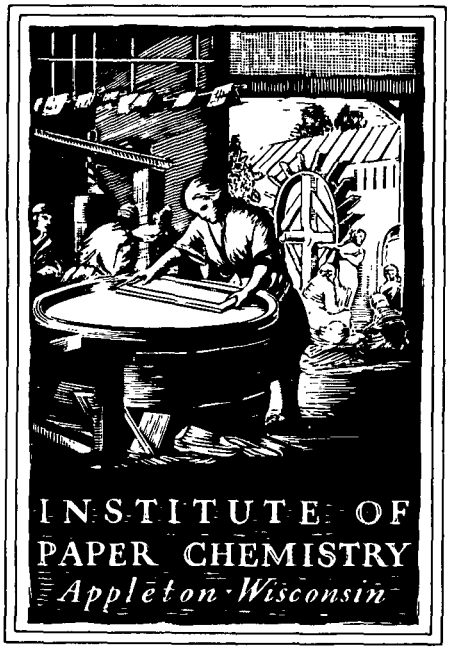
PROJECT ADVISORY COMMITTEE

Subcommittee on

Forest Genetics



IPC STAFF STATUS REPORTS

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Your advice and suggestions on any of the projects will be most welcome.

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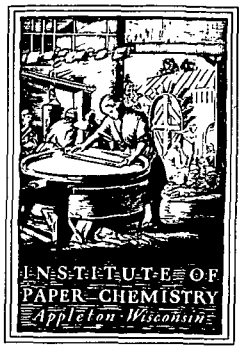


THE INSTITUTE OF PAPER CHEMISTRY
Post Office Box 1039
Appleton, Wisconsin 54912
Phone: 414/734-9251
Telex: 469289

February 23, 1988

TO: Members of the Forest Genetics PAC

As indicated in my recent letter, I am forwarding a detailed agenda and some advance reading material for the spring meeting of our Forest Genetics Project Advisory Committee.

Included in the reading material is a copy of our most recent status report as prepared for the Research Advisory Committee. The report is divided into three sections, reflecting planned changes in project number and nature for the coming fiscal year. We believe the documents both indicate our current status and accurately recount conclusions from the joint planning session in Atlanta last month.

To provide further detail about recent developments, I am enclosing a pre-print" of the summary from our forthcoming annual report. Bound copies of the complete report will be forwarded to you in the near future. The report is now being edited, and readied for printing. Please use these several items in preparing for participation in the March meeting.

We look forward to sharing new developments and discussing the future with you on March 30 and 31. Please remember to register if you have not already done so. Be certain also to share the enclosures with other members of your firm who are planning to participate.

Many thanks and best regards.

Sincerely,

Ron

Ronald J. Dinus
Director
Forest Biology Division

RJD/jmm
Enclosures

AGENDA

FOREST GENETICS PROJECT ADVISORY COMMITTEE

March 30-31, 1988
The Institute of Paper Chemistry
Continuing Education Center
Appleton, Wisconsin

Wednesday, March 30

8:00 am	Laboratories open & personnel available	
NOON	Lunch, CEC Dining Room	
1:00 pm	Opening Remarks Welcome/Introductions Overview Review of PAC Recommendations	Dinus
1:20	Initiation & Maintenance of Embryogenic Callus Loblolly Pine, Immature Explants Summer Collections, Latest Results Winter Collections, New Work Prognosis	Becwar
1:45	Norway Spruce, Mature Explants Cotyledons, Latest Results Prognosis & Recommendations	Wann
2:05	Embryo Development & Maturation Loblolly Pine, Summer Collections Recent Findings & Prognosis	Nagmani
	Biochemistry of Development	
2:25	Lipids, Isozymes, & Reductants	Johnson
2:50	Protein Composition	Feirer
3:10	Coffee Break	
3:40	Conversion to Seedlings Zygotic Embryos, Loblolly Pine	Wann
4:00	Discussion of Short Term Benefits Conifers & Hardwoods	Wann & Staff
4:40	Summary & Discussion	Dinus
5:00	Cocktails & Dinner, CEC Dining Room	
7:00	Open Discussion	

AGENDA (Contd.)

Thursday, March 31

7:30 am	Breakfast, CEC Dining Room	
8:00	Agenda for Morning	Chairman/Dinus
8:15	Discussion	Committee/Staff
9:45	Coffee Break	
10:00	Discussion/Deliberations	Committee/Staff
11:15	Closing Remarks	Chairman/Dinus
11:30	Adjournment/Lunch, CEC Dining Room	

NEXT MEETING: October 26 & 27, 1988

PROJECT TITLE: Mass Clonal Propagation of Improved Conifers

PROJECT STAFF: Becwar, Dinus, Feirer, Johnson, Nagmani, Verhagen, Wann

PRIMARY AREA OF INDUSTRY NEED: Raw Materials

Date: February 8, 1988
Budget: $500,000
Period Ends: 6/30/89
Project No.: 3223
Approved by VP:

PROGRAM GOAL: Assured and low-cost supplies of quality softwood fiber

PROJECT OBJECTIVE:

Develop reliable cell and tissue culture systems for mass clonal propagation of improved conifers.

PROJECT RATIONALE:

Major increases can be obtained in fiber production, quality, and uniformity via mass cloning of improved trees. Reliable cell and tissue culture systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, increased pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening for and selecting useful variants in culture could also lower costs and accelerate the pace of conventional tree breeding.

Improved growth will reduce raw material costs and increase returns on capital invested in land and equipment. Greater uniformity of clonal plantations can lower both woodlands and mill operating costs as well as enhance end-use properties. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

RESULTS TO DATE:

Past research on cell and tissue culture systems has brought somatic embryogenesis, one method of mass cloning, closer to commercialization. Embryogenesis in Norway spruce, our model system, is now controlled and reproducible. Initiation of embryogenic callus from developing and fully developed seed is straightforward. Similar progress has been made with white spruce, a commercially important species. The ability to use fully developed seed permits year-round experimentation. Embryo numbers can be quantified and Norway spruce seedlings have been recovered. Formation and proliferation of somatic embryos have also been observed in liquid suspension cultures. Though some seedlings have been recovered, development/maturation of somatic embryos and conversion to seedlings remains difficult. Current efforts are concentrated on increasing the efficiencies of these steps.

Somatic embryogenesis has also been obtained in our target species, loblolly pine and Douglas-fir, as well as several other pines and pitch X loblolly hybrids. Pine initiation frequencies remain low and variable, but results are reproducible and methods appear applicable to pines in general. Progress on embryo development in pine has been slow but steady. Embryogenic callus can be initiated in Douglas-fir, but not on a reliable basis. Initiation frequencies remain low, callus enlargement is slow, and embryo development has not yet been observed. Current work with the target species involves improving initiation frequencies and reliabilities, and securing embryo development.

PLANNED ACTIVITY FOR THE PERIOD:

Continue efforts to increase frequency and reliability of initiation in target species. Devise and/or refine protocols for improved embryo development and maturation, and for more efficient conversion of embryos to seedlings. Document course of development in zygotic embryos, and develop guideposts for manipulating somatic embryo development. Document interim or side benefits of tissue culture research; eg. techniques made available, tests for disease resistance, and other potential applications. Gradually expand work on liquid suspension cultures and initiation of embryogenic callus from more mature materials (eg., cotyledons). Add appropriate personnel, as authorized, to address these issues and to broaden project expertise. Use related student research to leverage staff efforts, as numbers and interest permit.

POTENTIAL FUTURE ACTIVITIES:

Continue exploratory work on origin of embryogenic callus and development of callus from protoplasts. Extend work to trees mature enough to have been proven genetically superior.

PROJECT TITLE: Mass Clonal Propagation of Genetically Improved/Engineered Hardwoods

PROJECT STAFF: Staff

PRIMARY AREA OF INDUSTRY NEED: Raw Materials

Date:	February 8, 1988
Budget:	$75,000
Period Ends:	6/30/89
Project No.:	New
Approved by VP:	

PROGRAM GOAL: Assured and low-cost supplies of quality hardwood fiber

PROJECT OBJECTIVE:

Develop reliable, low-cost systems for mass clonal propagation of genetically improved-engineered hardwoods.

PROJECT RATIONALE:

Major increases can be obtained in fiber production, quality, and uniformity via mass cloning. Reliable cloning systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, greater pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening/selection for useful variants in tissue culture holds promise for raising the pace and efficiency of conventional tree breeding.

Accelerated growth will ensure reliable raw material supplies, reduce their costs, and raise returns on capital invested in land and equipment. Greater uniformity can lower both woodlands and mill operating costs as well as enhance properties related to end-use performance. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

RESULTS TO DATE:

This is a new project. Some work on hardwoods has been done at the Institute over the years, but largely on an exploratory basis. Results from this work and that of other organizations indicate that hardwoods can be manipulated with relative ease. Other exploratory work at the Institute has suggested that tissue culture can be used to test for disease resistance. Work elsewhere infers that novel variants produced in culture can be isolated and used to introduce new and different traits into breeding stock and clonal reforestation programs.

PLANNED ACTIVITIES FOR THE PERIOD:

Given the developments noted above, this new project will seek to develop commercial systems for mass clonal propagation of selected hardwoods. Early activities include: prepare "white paper" comparing fiber properties, growth rates, suitabilities for plantation management, and propensities for clonal propagation of important species, and recommending species to use in future research. Document interim or side benefits of cloning research. Begin evaluating available and new cloning methods, adapting them to important species, and combining them into cost effective systems for application. Add personnel with appropriate skills, as authorized.

POTENTIAL FUTURE ACTIVITIES:

Extend work to trees mature enough to have been proven genetically superior. Explore novel methods for hastening genetic improvement by testing and early selection in culture. Develop cell culture systems suitable for genetic engineering.

PROJECT TITLE: Biochemistry of Clonal Propagation

Date: February 8, 1988
Budget: $150,000
Period Ends: 6/30/89
Project No.: New
Approved by VP:

PROJECT STAFF: Feirer, Johnson

PRIMARY AREA OF INDUSTRY NEED: Raw Materials

PROGRAM GOAL: Assured and low-cost supplies of quality softwood and hardwood fiber

PROJECT OBJECTIVE:

Develop an improved understanding of biochemical mechanisms controlling embryogenesis and other cloning methods, and devise procedures for raising the effectiveness and efficiency of mass cloning methods.

PROJECT RATIONALE:

Improved understanding of biochemical mechanisms controlling embryogenesis and other cloning methods will shorten the time to commercial application of clonal forestry, raise their efficiencies, and facilitate extension to trees mature enough to have been proven genetically superior.

RESULTS TO DATE:

As a result of past Institute efforts, somatic embryogenesis in Norway spruce, our model system, is now controlled and reproducible. Initiation of embryogenic callus is straight-forward; embryo numbers can be quantified and seedlings have been recovered. Somatic embryogenesis has also been obtained in our target species, loblolly pine and Douglas-fir, but initiation frequencies remain low and seedlings have not been recovered.

Earlier and ongoing work on the biochemistry of embryogenesis in Norway spruce has yielded useful data on differences between embryogenic and nonembryogenic cultures, and some knowledge of factors affecting embryogenesis. Such differences and associated markers are being used to screen cultures for embryogenic potential, and monitor the effects of modified or new protocols for callus initiation and embryo development in our target species. Techniques for isolating, purifying, and characterizing proteins, enzymes, RNA, and DNA have been developed or refined. With further refinement, these can be used to increase the reliabilities and frequencies of somatic embryo initiation, development, and conversion to seedlings.

PLANNED ACTIVITY FOR THE PERIOD:

Despite the advances noted above, knowledge of the mechanisms limiting various cloning methods, especially in tissues derived from trees mature enough to have been proven genetically superior, remains fragmentary. As a result, methods

for increasing the effectiveness and efficiency of cloning processes are not available. This new project will concentrate available personnel on developing such methods.

ACTIVITIES OVER THE NEAR-TERM INCLUDE:

Continue using biochemical markers and analyses to characterize the embryogenic potential of cultures and facilitate initiation of embryogenic callus. Accumulate baseline data on development of zygotic embryos, and use results to identify and manipulate factors limiting somatic embryo development and conversion to seedlings.

Use related student research to leverage staff efforts, as numbers and interest permit.

POTENTIAL FUTURE ACTIVITIES:

Document differences between mature and immature tissues, and devise means for rendering mature tissues more easily manipulated in culture. Refine and apply methods for certifying genetic fidelity of seedlings derived from cloning of softwoods and hardwoods. Refine and develop promising new techniques and analyses. Explore techniques for early selection and testing.

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

MASS PRODUCTION OF CONIFER HYBRIDS

SUMMARY

Emphasis in 1987 was concentrated more on our target species than in earlier years. Having found initiation in Norway spruce, our model system, rather straightforward, increased attention was given to obtaining embryogenesis in the target species. Greater effort was also devoted to embryo development/ maturation and conversion. Work in the biochemistry area moved even more toward direct support of cell and tissue culture activities.

INITIATION OF EMBRYOGENIC CALLUS

The year was a landmark one for initiation. Our first embryogenic loblolly pine cultures were obtained early in the year. They have been maintained without great difficulty, and others have since been initiated. Overall frequencies have been low, but several open-pollinated families proved quite responsive and the best averaged five percent across treatments and lines. All told, embryogenic lines were obtained from 7 of the 10 families used in the experiments. Viewed another way, initiation occurred with explants from three separate collections, including those made in the U.S. during summer, 1986, from South America in winter, 1987, and from the U.S. in summer, 1987. Our protocol thus yielded reproducible results, and worked with explants from a number of genetic backgrounds. We were also able to identify optimal explant developmental stage. This more precise definition of explant stage will increase efficiency of future work on initiation - fewer collections, lightened workloads, and more time for experimentation on other fronts.

The year also saw initiation of embryogenic callus in pitch X loblolly pine hybrids. To our knowledge, this is the first such report for a conifer hybrid. Mass cloning from even hybrid embryos would have considerable value because of the difficulty and expense of producing commercial quantities of hybrid seed by conventional means.

One hybrid line is highly vigorous, producing numerous early stage embryos with clear potential for development. Once this and the most active loblolly lines have been increased, we will use them in experiments on embryo development/maturation. At that time, we also would be pleased to offer samples to Member Companies for research in their own laboratories. In the meantime, we are using the results to frame new ways to increase initiation frequencies.

Efforts to induce embryogenesis in Douglas-fir intensified in 1987, with particular emphasis on explant developmental stage and media composition. Several test media produced translucent white callus, phenotypically similar to embryogenic spruce callus. Interestingly, such callus originated from different tissues depending upon explant developmental stage. Some calli produced cell types and proembryos resembling those in spruce, and results of biochemical assays were similar to those for embryogenic callus of other species. All cultures have since turned green or brown, and reproducible embryogenesis still eludes us, at least for the present. That is, some green cultures may yet prove embryogenic. One such culture, initiated in 1985, became embryogenic about 18 months later. Viewed as a whole, these results suggest that we are close to having the proper explant, and to triggering its potential. Further protocol refinements are needed, and we are considering the differential origin and sprucelike behavior in designing new experiments.

In keeping with near-term plans, efforts to develop additional biochemical markers have ceased, and results have been summarized for publication. Assays of the six species for which we have embryogenic and nonembryogenic callus show that most available markers clearly separate the two callus types. Indeed, one marker, total reductants, successfully predicted embryogenic potential of loblolly and pond pine cultures before appearance of somatic embryos. Similarity of responses among species suggests that embryogenesis is governed by a common set of metabolic reactions, and that the greatest utility of our markers is yet to be realized - provision of clues to critical mechanisms. Developing an appreciation for these may enable us to start or stop the correct ones at appropriate times.

DEVELOPMENT/MATURATION

The exciting news for the year about initiation was supplemented by modest progress toward assured and predictable embryo development. Considerable effort was devoted to empirical as well as biochemical studies of development. Included were several experiments evaluating factors leading to improved development in white spruce, and their impact on development in loblolly pine. A combination of abscisic acid and higher sucrose concentration clearly stimulated embryo development in both species. The two substances had complementary effects, and their utility varied with when they were applied and how long they were retained in the media.

At the time of treatment, white spruce calli were further advanced than pine, and the best media moved the former much further along. Indeed, embryogenesis was completed in white spruce; many embryos reached the cotyledon stage and one seedling was transferred to soil. Pine development stopped before the cotyledon stage, but nevertheless did progress farther than in earlier work.

Different callus lines varied in response to treatment, and no one treatment affected all lines the same. This outcome may be a genetic effect, but more likely results from individual calli not initially being at the same stage of development. To offset this, we are working to improve our understanding of such variation, of what stages are receptive to manipulation, of how long each stage remains responsive to treatment, and of what treatments are needed when embryos move to yet another stage.

In another approach to development, an attempt was made to develop immature zygotic embryos of loblolly pine in culture and convert them to seedlings. Immature embryos at several stages of development were cultured on different media, with and without various nutrients and extracts of female gametophytes. Results showed that precotyledonary embryos could not be grown into plants, and only half or less reached the cotyledon stage. Development stopped even on our best media. Combining leads from these experiments with results from earlier work on somatic embryos nevertheless provided much information about the stage at which development stops and about the kinds of media changes that are needed. In contrast, zygotic embryos just beyond the cotyledonary stage readily elongated, regardless of culture conditions, and many were rooted and transferred to soil. Thus, techniques available for conversion to seedlings, though not optimal, are reasonably workable. Improving their efficiency should be relatively straightforward, and easier than perfecting the methods needed for early development.

To aid and abet the empirical approaches described above, we are also endeavoring to understand the physiology and biochemistry of embryo development. Typical approaches involve monitoring appearance and/or disappearance of proteins, lipids, and other compounds during development of zygotic and somatic

embryos. Knowing patterns normal for zygotic embryos may enable us to better steer somatic embryo developoment.

Along these lines, methods for examining protein patterns were refined for application to conifer systems, and used to follow protein accumulation in developing zygotic embryos of loblolly pine. Embryos in six different stages of development were available from cones collected for initiation experiments. A number of proteins were conspicuous by virtue of gradual accumulation over the summer, several were found to appear suddenly at later stages, and a few appeared during mid-development and disappeared later. Results mirrored those observed in a number of Angiosperms, where both protein patterns and development have been linked to changes in concentration of abscisic acid. Such findings underscore the merits of using tools, such as abscisic acid and media osmolarity, to further development of somatic embryos.

Earlier observation of differences in the number and size of lipid bodies in electron micrographs of zygotic and somatic embryos prompted work on patterns of lipid accumulation in embryogenic calli, developing embryos, and fully developed seeds. Premliminary results indicate that both embryos and female gametophytes from fully developed seed have similar lipid composition. Of greater interest, a polar lipid compound was found in embryogenic spruce callus, but not in nonembryogenic callus. This same or a similar lipid was later found in early stage zygotic embryos of loblolly pine. Detailed comparisons of developing zygotic and somatic embryos are underway to determine if this or related compounds can be used to track or promote development.

In a similar vein, an effort was made to capitalize on earlier demonstration of parallels between glutathione biosynthesis and total reductants

content and development of zygotic embryos of red and white pines. These parameters along with activity of the enzyme, glutathione reductase, were examined in developing zygotic embryos and female gametophytes of Douglas-fir and loblolly pine. Changes in glutathione and total reductants content of Douglas-fir varied much as in the earlier pine work. Observations in loblolly differed somewhat, possibly as a result of delayed seed development occasioned by drought. Patterns of enzyme activity in loblolly paralleled those of glutathione, suggesting a role in regulating reduced glutathione content. Such findings are helping determine when and how to use tools, such as inhibitors of glutathione metabolism, to foster development of somatic embryos.

CONVERSION AND FIDELITY/PERFORMANCE

"Somatic seedlings" produced in 1986 figured in several experiments this year and some new "seedlings" are being readied for transfer to the greenhouse. Results to date indicate that Norway spruce lines used in the experiments produced mature embryos at frequencies varying from 1 to 15 percent. With our best treatments, 56 percent of mature embryos initiated root growth, and 29 percent of these survived transfer to and growth in the greenhouse. Though overall recovery was low, the experiments improved our understanding of the individual process steps and provided several useful leads.

Surviving plants are phenotypically similar to and behave like regular spruce seedlings. They set buds, went dormant over winter, and initiated spring growth in synchrony with their zygotic counterparts. Growth during 1987 roughly equalled that of controls. This is the first demonstration of normal growth and behavior of such plants across seasons.

Some preliminary work on genetic fidelity was also done, mainly in the interest of testing and refining isozyme analyses. First results confirmed, as expected, that somatic "seedlings" of the same clone and developmental status had identical isozyme patterns. Zygotic seedlings, in contrast, had variable patterns. Perhaps the most significant outcome was enhanced sensitivity of the techniques. Isozyme patterns can be detected for single needles and even individual embryos. Since some isozyme patterns vary with developmental stage, the techniques should be useful for tracking development of zygotic and somatic embryos, and identifying ways to improve protocols for development.

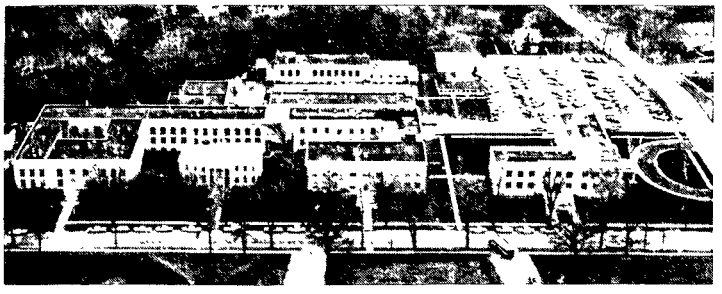
EXPLORATORY RESEARCH

Efficiency of mass cloning would be increased by systems that produce large numbers of somatic embryos, rapidly and with mimimal labor. Cell suspensions are an attractive alternative in these regards, and exploratory work with Norway spruce suspensions paid significant dividends in 1987. Embryo proliferation was rapid, with embryo numbers peaking at roughly 100 per ml of suspension after only 14 days. Proliferation continued when subculturing was done at 10 to 12 day intervals. Manipulations attempted to date, regrettably, did not foster complete embryo development, but several promising tacks, including sequential changes in composition of suspension media, are being pursued.

Protoplast cultures could provide an ideal system for mass cloning, genetic transformation, and generation of novel variants. Exploratory work aimed at developing a workable protoplast system continued this year with cells from our successful Norway spruce suspension cultures. A variety of methods for protoplast isolation and culture were tested. The best gave significant yields of viable protoplasts, with roughly 50 percent remaining viable for use in

further experiments. About five percent divided at least once, and a small fraction continued dividing to form small cell clusters. Given this degree of progress, we are continuing to explore means for fostering division and obtaining callus.

Also on the exploratory front, availability of sweetgum shoot cultures provided an opportunity for attempting genetic transformation. The work was successful, and marks the first introduction and expression of foreign genes in a commercially important southern hardwood species. In addition, methods used to confirm transformation will be useful in our main effort on somatic embryogenesis.



THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

FOREST GENETICS PROJECT ADVISORY COMMITTEE

HANDOUTS

March 30-31, 1988

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NEXT MEETING: October 26 & 27, 1988

FOREST GENETICS
Project Advisory Committee

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FOREST GENETICS PROJECT ADVISORY COMMITTEE

The Institute of Paper Chemistry
Appleton, Wisconsin

March 30-31, 1988

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Mr. Robert Lazar
Union Camp Corporation

Mr. Gregory N. Leach
Champion International Corporation

Mr. Billy Wayne McIntyre
MacMillan Bloedel Inc.

Mr. Sharon R. Miller
Chesapeake Corporation

Mr. Edwin G. Owens
Westvaco Corporation

Dr. Gerald Pullman
(substituting for Rex McCullough)
Weyerhaeuser Company

Mr. Martin P. Rech
Consolidated Papers, Inc.

Dr. Ronald A. Woessner
The Mead Corporation
Georgia Kraft Division

Guests

Dr. David Canavera
Westvaco Corporation

Mr. Don Hubbard
Leaf River Forest Products

Mr. Joseph Weber
Simpson Timber Company

IPC Personnel

Mike Becwar
John Carlson
Jud Conkey
Terry Conners
Ron Dinus
Russ Feirer
Debra Hanson
Morris Johnson
Lynn Kroll
Nagmani Rangaswamy
Shirley Verhagen
Steve Wann
Gary Wyckoff
Judy Wyckoff
Richard Matula
Wendall Smith
Ronald Yeske

CODES

Tissue response and the results of many studies may be altered or complicated by the genetic differences between cell lines and/or the length of time in culture. To aid the reader (reviewer) in understanding, and the investigator in reporting/analyzing, it is important to be aware of the tissue source used for each study. An example and explanation of our standard tissue identification coding system is presented below; however, at times only part of the code may appear in a text.

All cell lines in excess of one year old:

Example: 20(NS 384-1)2E

20 = subcultured 20 times

NS = Norway spruce

384 = research plan (RP384)[a]

-1 = time of initiation or treatment identification

2 = line or genetic source, e.g., seedling No. 2

E = Immature embryo; explant type (only used if cell line derived from more than one explant within a research plan).

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[a]Each experiment initiated by any team member has an approved research plan with an identifying number. The tissue source origin (clone, seed lot, etc.) and initiation date is recorded under that number in the investigator's IPC research notebook and is available in the Tissue Culture Research Plan files.

Cell lines less than one year old from immature cone collections:

Example: 5(LP6B)E - the RP No. is deleted and the letter within parentheses indicates cone source code.

Species Codes	Explant Codes
LP - loblolly pine	C = cotyledon
DF - Douglas-fir	H - hypocotyl
PP - pitch pine	B - bud
PO - pond pine	E - immature embryo
NS - Norway spruce	M - mature embryo
WP - white pine	N - nucellus
WS - white spruce	G - gametophyte

CONE SOURCES - 1987

Species	Tissue Culture Code	Source	Industrial Codes
Douglas-fir	DF J	Weyerhaeuser	WTC-357
	DF K	Federal Way, WA	WTC-358
	DF L		WTC-359
	DF M		WTC-360
	DF N		WTC-361
Loblolly pine	LP A	Union Camp	10-1003 D-22 HQI
	LP B	Rincon, GA	10-1007 F-21 HQI
	LP C		10-1011 C-20 HQI
	LP D		10-1018 B-16 HQI
	LP E		10-1019 C-14 HQI
	LP F	Westvaco	7-34
	LP G	Summerville, SC	7-56
	LP H		11-9
	LP I		11-10
	LP J		11-16
	LP K	Rigesa	7-34[a]
	LP L	Tres Barras, Brazil	7-56[a]
	LP M		11-10[a]
	LP N		11-16[a]
	LP O		11-19[a]
	LP P		11-9[a]
	LP R	Westvaco Summerville, SC	11-25
	LP S	Rigesa	6
	LP T	Tres Barras, Brazil	18
	LP U		22
Norway spruce	NS	Greenville, WI	--
	NSA	U. Arkansas Fayetteville, AR	
	SHNS	Shiocton, WI	
White spruce	WS A	Greenville, WI	--
	WS B		--
	WS 65	Oconto River Seed Orchard, WI	
Pitch/loblolly Hybrid	PL	Westvaco Summerville, SC	65 x LP

[a]Cones obtained from progeny of the given clone.

STATISTICS

Where statistics beyond means and standard deviations (S.D.) were used in the evaluation of results to be presented, the data were subjected to analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test for multiple comparison of means. Values with a common superscript letter are not significantly different from each other ($P < 0.05$). The number of replications is indicated by N.

INTRODUCTORY REMARKS - RON DINUS

WELCOME

NEW FACES - MEMBERS AND GUESTS

OVERVIEW

- PROJECT BACKGROUND
- RECENT DIRECTIONS

INSTITUTE AND INTERNAL AFFAIRS

PAC RECOMMENDATIONS

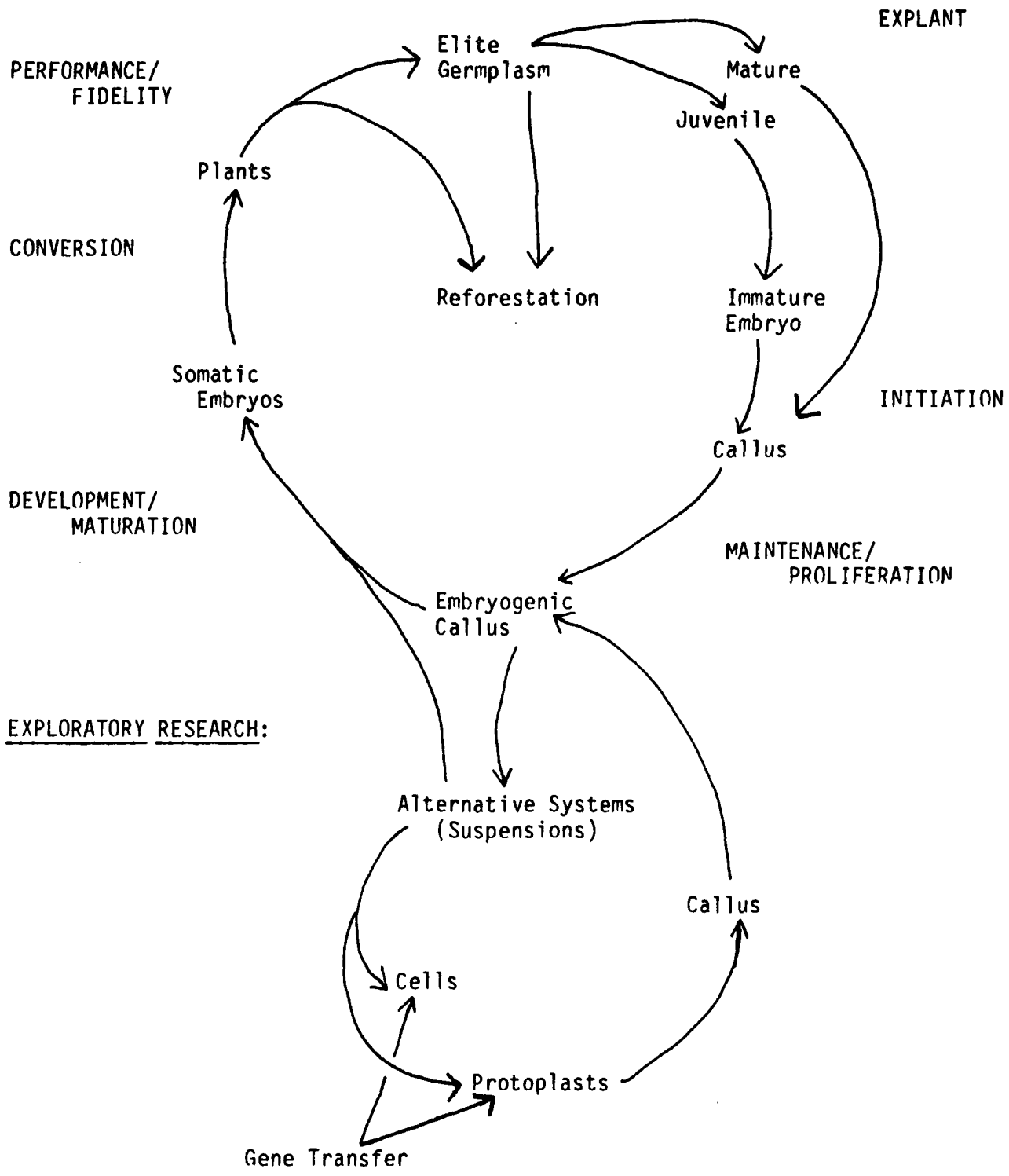
GROUNDRULES AND ANNOUNCEMENTS

THANKS, AND LET'S HEAR SOME RESULTS

PAC RECOMMENDATIONS

ISSUE	ACTION OR PLAN
EMPHASIZE TISSUE CULTURE, SUPPORT WITH BIOCHEMISTRY	ACCUMULATING BASELINE DATA; WILL USE AS GUIDES, & CONSIDER MECHANISMS
MODEL SYSTEM	KEEP NS, CURTAIL OTHERS. SECURE MATERIAL, STUDY PROCESS STEPS, REFINE, ADJUST, & APPLY TO TARGETS
ENHANCE PROGRAM REPUTATION	APPRECIATE ENCOURAGEMENT, CONTINUING TO EMPHASIZE
CHARTING EFFORT & PROGRESS	THANKS, WORKING TO ENLARGE & REFINE. SUMMARY REMARKS
OBTAINING EXTERNAL FUNDING	WILL USE SUGGESTIONS; STRESS DEVELOPMENT/MATURATION
DEVELOPMENT/MATURATION & CONVERSION = KEY ISSUES	LARGEST SINGLE EFFORT, CONTINUING ZYGOTIC/SOMATIC COMPARISONS. ALSO RECRUITING EMPLOYEES & SEEKING OUTSIDE HELP
GENETIC CONTROL	N SPRUCE - REPEAT OLD AND NEW FAMILIES + INITIATE FROM COTYLEDONS. TARGET SPP - ORCHARD HALF-SIBS
EXPLORATORY	CONTINUING AS BEFORE, BUT USING STUDENTS AS WELL
COMMUNICATION	TRYING TO WORK MORE WITH CHAIR. DISCUSS TEAM ISSUES & NEED TO STREAMLINE SYSTEM

MASS PROPAGATION OF IMPROVED CONIFERS



INSTITUTE AND INTERNAL AFFAIRS

****

INSTITUTE ADMINISTRATION

MEMBERSHIP

MID-RANGE PLAN

CLASS OF 1988

MIGRATION OF 1991

****

PROJECT PERSONNEL

VISITORS

STUDENTS

MEETINGS AND CONFERENCES

****

Mead

December 14, 1987

Dr. Ronald J. Dinus
The Institute of Paper Chemistry
P.O. Box 1039
Appleton, WI. 54912

Dear Ron:

The presentations made at the Project Advisory Committee meeting of October 26 and 27, 1987, were well prepared and informative to the members. Progress clearly had been made in several areas. The emphasis on tissue culture with biochemistry support continued as had been requested by previous PAC's. The species emphasis had the right trend working with the spruces as model species with the hard pines being the primary target. The Norway spruce-white spruce issue needs some thought. You have continued to enhance the visibility of the Institute program through publications, presentations, visits to other locations, and by receiving visitors at the Institute. The presence of Dr. Stan Krugman and Dr. Ralph Mott clearly demonstrated this effort. Visitors with this stature greatly enhance such meetings.

Your response to our request for details on where effort was expended since the last PAC meeting (attachment 1) was what we had requested. The flow diagram you presented (attachment 2) can also serve to chart progress from meeting to meeting after some enhancement. The use of these tools on a continuing basis is strongly endorsed by the committee.

The necessity of obtaining grant funding was one topic which generated significant discussion. Specific recommendations regarding this are as follows:

1. Call together a working group, including individuals with a proven track record in successul grants and/or reviewing grants, with the expressed objective of identifying areas of research at IPC (or associated programs) with a good probability of receiving grant funding.

2. Identify university programs with a proven track record in successful grants, who are working in related areas to IPC's highest priority objectives, and pursue joint grant proposals.

Two suggested areas to pursue grants are the physiology of developing somatic embryos and the role of hormones in plant development. The team surely has many additional ideas.

The failure of most somatic embroys to not go beyond the cotyledon expansion stage pinpoints the need for additional research in this area. Some questions which arose in regard to this were 1. Is dormancy involved?, 2. Is drying needed?, 3. Are the embryos being fed with the right carbon sources through the cotyledons? Another area of suggested research was the need for more information on the environment surrounding the zygotic embryo in the archegonium of the female gametophyte. Understanding of hormone contents, sugar, lipid, nutrient, and oxygen levels might be necessary.

Some breakdown in communication occured between yourself and PAC regarding the 5-year-plan. The committee felt items of this nature could be avoided if you were to call on the PAC chairman for assistance and clarification when you felt the need.

The exploratory work on sweetgum and agrobacterium again demonstrated that techniques that work with other species work with trees.

Much effort went into the research results presented at this meeting. The committee thanks you and the team for their continuing efforts in a very exciting but difficult area of research.

Some additional comments provided by several members are attached.

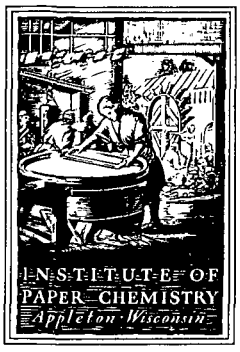
Sincerely,

Ron Woessner

Ron Woessner

RW/gsp
Attachments

cc: PAC Committee Members



THE INSTITUTE OF PAPER CHEMISTRY
Post Office Box 1039
Appleton, Wisconsin 54912
Phone: 414/734-9251
Telex: 469289

January 25, 1988

Dear Ron and PAC Members:

Thank you for your response to our fall meeting. We appreciate your comments as a long-time PAC member, and welcome the views of other members.

Your remarks concerning enhanced visibility of our program are especially appreciated. We have made much progress on this front, but still have room for improvement. We will, therefore, continue to seek opportunities for invited papers at conferences, presentations to a wider array of audiences, and timely publication in referred journals. Directed cooperation with other organizations also deserves high priority. Having guests at the fall meeting has paid a variety of dividends, and we intend to continue the practice.

Special thanks for your kind compliment on our process diagram and description of how much effort was expended where. In line with your suggestions, we will refine the diagram, and attempt to incorporate information on both progress and effort. With time, we hope to illustrate our own status and that of other organizations active in the field. An effort will be made to include such charts in the reading material forwarded before each meeting.

We recognize the necessity of external funding, and value your suggestions on ways to improve our abilities to win grants and contracts. We are fortunate to have several past and present PAC members who have been involved in all phases of preparing and evaluating proposals. Their familiarity with the process as well as our project make them a valuable resource. The team, however, feels that some time and patience are required. We are rather new to the game, and must also labor to overcome prejudices among our peers in "traditional universities." Some bias has been evident in reviews of our proposals. Though generally favorable from a scientific standpoint, the reviews contained objections to providing federal funds to a "non-traditional university," especially one perceived as already well-supported by industry. Dividing the current project into several separate efforts, each with its own budget, may offset some such criticism. Rest assured, then, that we will aggressively seek ways to augment member support.

We are glad the committee recognizes the roadblock posed by embryo development/maturation. This step historically has been the most critical one regardless of species, and we concur that a novel approach is needed. We now have a fair understanding of external factors (eg., ABA and osmoticum) that can be manipulated to aid development, and the team feels that an empirical approach to manipulation should be pursued in cell lines showing strong embryogenic capabilities. To further empirical efforts, we advocate accumulation of biochemical and anatomical data on development of both somatic and zygotic embryos. Biochemical and anatomical events, noted in the natural course of zygotic embryogenesis, can be used as guides for keeping somatic embryos on course. Such events or markers could be used to identify those stages in which somatic embryos depart from the norm. When a difficult stage is encountered, external manipulations could be tried and the markers used as feedback to determine treatment efficacy. Thus, we wish to make zygotic/somatic comparisons when possible in Norway spruce and loblolly, and test promising treatments on somatic embryos. More fundamental understanding of conifer embryo development could well be an attractive topic for a grant proposal.

Some committee members had specific questions or comments, and we wish to address several such issues. Concerning species, the team is pleased that members endorse use of a model system, namely Norway spruce. This species can be used to model every step, beyond initiation, in the process. The Norway system, however, is not functioning as effectively as desired, and we feel that a rededication of effort is necessary. Included would be better documentation of difficult steps. Work on Norway spruce development/maturation and conversion is particularly important as this is the only species in which comparisons can be made between all stages of somatic and zygotic embryogenesis.

Efforts with loblolly pine will focus on:

1. Increasing initiation frequencies in those half-sib families that have a propensity for initiation and/or have an embryogenic phenotype resembling that of Norway spruce (ie., embryos are visible in callus even when cultured on proliferation medium);

2. attempting to grow precotyledonary zygotic embryos into plants; and

3. stimulating development/maturation in available lines.

To concentrate more on Norway spruce and the target species, work on white pine, pond pine, and white spruce will be curtailed. Such efforts seem ancillary to our main goals. In loblolly pine, attention will focus on lines showing propensity for development, and producing sufficient numbers of embryos for meaningful study. Currently, only two or three pine lines and

one pitch X loblolly hybrid line fit this criteria. In Douglas-fir, efforts will concentrate on increasing reliability and frequency of initiation. Work on fidelity will be delayed until a more meaningful population of plants is available.

Several members expressed concern about genetic control. In Norway spruce, we have continued access to the same half-sib families used to establish successful lines, and can use seed from them for comparisons of somatic and zygotic plants. In addition, the distinct opportunity exists, and is being pursued, to initiate embryogenic callus from cotyledons. Donor plants can be saved, thus allowing for exact genetic comparison of somatic and zygotic materials.

In loblolly, we would like to work with full-sib material. We, nevertheless, feel it best to continue with half-sib material of known (seed orchard) origin. Recall that full-sib seed is limited in availability, we have very low initiation frequencies, and that our understanding of genotypic effects is poor. Several clones have shown higher initiation frequencies than others for two consecutive years, but we prefer sampling a broad genetic base in the interest of developing a generally applicable technology.

In accordance with other comments, research on such topics as suspension cultures, mature explants, portoplasts, and gene transfer will continue on an exploratory basis. Several students are interested in these topics, and we can use their talents to leverage staff efforts. Also, the current round of suspension culture experiments will soon be completed, and we plan to submit a paper for publication soon thereafter. Similarly, results from work on gene transfer in sweetgum have been summarized and forwarded to a major journal.

Your recommendation to work more closely with the chairman is well-taken. The speed with which we resolved the subculturing and mucilage issues last fall is a prime example of productive interaction. We would appreciate the opportunity to place more such issues, along with topics of concern to the Institute, on future agendas. In addition, the team wishes to devote a significant portion of each meeting to "joint" problem-solving and planning. This would help bring all of us to a common level of understanding, promote free-wheeling debate, and capture the best from staff/member interaction.

In closing, we look forward to continued assistance from you, and to visiting with you in March.

Sincerely,

Ronald J. Dinus
Director, Forest Biology Division
** For Team 3223 **

RJD/jml

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

Status Report

to the

FOREST GENETICS

PROJECT ADVISORY COMMITTEE

Project 3223

THE MASS PRODUCTION OF CONIFERS

March 30-31, 1988

PROJECT TITLE: Mass Clonal Propagation of Improved Conifers

PROJECT STAFF: Becwar, Dinus, Feirer, Johnson, Nagmani, Verhagen, Wann

PRIMARY AREA OF INDUSTRY NEED: Raw Materials

Date: February 8, 1988
Budget: $500,000
Period Ends: 6/30/89
Project No.: 3223
Approved by VP:

PROGRAM GOAL: Assured and low-cost supplies of quality softwood fiber

PROJECT OBJECTIVE:

Develop reliable cell and tissue culture systems for mass clonal propagation of improved conifers.

PROJECT RATIONALE:

Major increases can be obtained in fiber production, quality, and uniformity via mass cloning of improved trees. Reliable cell and tissue culture systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, increased pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening for and selecting useful variants in culture could also lower costs and accelerate the pace of conventional tree breeding.

Improved growth will reduce raw material costs and increase returns on capital invested in land and equipment. Greater uniformity of clonal plantations can lower both woodlands and mill operating costs as well as enhance end-use properties. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

RESULTS TO DATE:

Past research on cell and tissue culture systems has brought somatic embryogenesis, one method of mass cloning, closer to commercialization. Embryogenesis in Norway spruce, our model system, is now controlled and reproducible. Initiation of embryogenic callus from developing and fully developed seed is straightforward. Similar progress has been made with white spruce, a commercially important species. The ability to use fully developed seed permits year-round experimentation. Embryo numbers can be quantified and Norway spruce seedlings have been recovered. Formation and proliferation of somatic embryos have also been observed in liquid suspension cultures. Though some seedlings have been recovered, development/maturation of somatic embryos and conversion to seedlings remains difficult. Current efforts are concentrated on increasing the efficiencies of these steps.

Somatic embryogenesis has also been obtained in our target species, loblolly pine and Douglas-fir, as well as several other pines and pitch X loblolly hybrids. Pine initiation frequencies remain low and variable, but results are reproducible and methods appear applicable to pines in general. Progress on embryo development in pine has been slow but steady. Embryogenic callus can be initiated in Douglas-fir, but not on a reliable basis. Initiation frequencies remain low, callus enlargement is slow, and embryo development has not yet been observed. Current work with the target species involves improving initiation frequencies and reliabilities, and securing embryo development.

PLANNED ACTIVITY FOR THE PERIOD:

Continue efforts to increase frequency and reliability of initiation in target species. Devise and/or refine protocols for improved embryo development and maturation, and for more efficient conversion of embryos to seedlings. Document course of development in zygotic embryos, and develop guideposts for manipulating somatic embryo development. Document interim or side benefits of tissue culture research; eg. techniques made available, tests for disease resistance, and other potential applications. Gradually expand work on liquid suspension cultures and initiation of embryogenic callus from more mature materials (eg., cotyledons). Add appropriate personnel, as authorized, to address these issues and to broaden project expertise. Use related student research to leverage staff efforts, as numbers and interest permit.

POTENTIAL FUTURE ACTIVITIES:

Continue exploratory work on origin of embryogenic callus and development of callus from protoplasts. Extend work to trees mature enough to have been proven genetically superior.

PROJECT TITLE: Mass Clonal Propagation of Genetically Improved/Engineered Hardwoods

PROJECT STAFF: Staff

PRIMARY AREA OF INDUSTRY NEED: Raw Materials

Date: February 8, 1988
Budget: $75,000
Period Ends: 6/30/89
Project No.: New
Approved by VP:

PROGRAM GOAL: Assured and low-cost supplies of quality hardwood fiber

PROJECT OBJECTIVE:

Develop reliable, low-cost systems for mass clonal propagation of genetically improved-engineered hardwoods.

PROJECT RATIONALE:

Major increases can be obtained in fiber production, quality, and uniformity via mass cloning. Reliable cloning systems will also open the way for genetic engineering and production/delivery of new genetic combinations having exceptional growth, greater pest resistance, special fiber properties, and enhanced site and/or climatic adaptability. Screening/selection for useful variants in tissue culture holds promise for raising the pace and efficiency of conventional tree breeding.

Accelerated growth will ensure reliable raw material supplies, reduce their costs, and raise returns on capital invested in land and equipment. Greater uniformity can lower both woodlands and mill operating costs as well as enhance properties related to end-use performance. Better or new fiber properties can improve end-use performance and foster development of value-added or new products.

RESULTS TO DATE:

This is a new project. Some work on hardwoods has been done at the Institute over the years, but largely on an exploratory basis. Results from this work and that of other organizations indicate that hardwoods can be manipulated with relative ease. Other exploratory work at the Institute has suggested that tissue culture can be used to test for disease resistance. Work elsewhere infers that novel variants produced in culture can be isolated and used to introduce new and different traits into breeding stock and clonal reforestation programs.

PLANNED ACTIVITIES FOR THE PERIOD:

Given the developments noted above, this new project will seek to develop commercial systems for mass clonal propagation of selected hardwoods. Early activities include: prepare "white paper" comparing fiber properties, growth rates, suitabilities for plantation management, and propensities for clonal propagation of important species, and recommending species to use in future research. Document interim or side benefits of cloning research. Begin evaluating available and new cloning methods, adapting them to important species, and combining them into cost effective systems for application. Add personnel with appropriate skills, as authorized.

POTENTIAL FUTURE ACTIVITIES:

Extend work to trees mature enough to have been proven genetically superior. Explore novel methods for hastening genetic improvement by testing and early selection in culture. Develop cell culture systems suitable for genetic engineering.

PROJECT TITLE: Biochemistry of Clonal Propagation

Date: February 8, 1988
Budget: $150,000
Period Ends: 6/30/89
Project No.: New
Approved by VP:

PROJECT STAFF: Feirer, Johnson

PRIMARY AREA OF INDUSTRY NEED: Raw Materials

PROGRAM GOAL: Assured and low-cost supplies of quality softwood and hardwood fiber

PROJECT OBJECTIVE:

Develop an improved understanding of biochemical mechanisms controlling embryogenesis and other cloning methods, and devise procedures for raising the effectiveness and efficiency of mass cloning methods.

PROJECT RATIONALE:

Improved understanding of biochemical mechanisms controlling embryogenesis and other cloning methods will shorten the time to commercial application of clonal forestry, raise their efficiencies, and facilitate extension to trees mature enough to have been proven genetically superior.

RESULTS TO DATE:

As a result of past Institute efforts, somatic embryogenesis in Norway spruce, our model system, is now controlled and reproducible. Initiation of embryogenic callus is straight-forward; embryo numbers can be quantified and seedlings have been recovered. Somatic embryogenesis has also been obtained in our target species, loblolly pine and Douglas-fir, but initiation frequencies remain low and seedlings have not been recovered.

Earlier and ongoing work on the biochemistry of embryogenesis in Norway spruce has yielded useful data on differences between embryogenic and nonembryogenic cultures, and some knowledge of factors affecting embryogenesis. Such differences and associated markers are being used to screen cultures for embryogenic potential, and monitor the effects of modified or new protocols for callus initiation and embryo development in our target species. Techniques for isolating, purifying, and characterizing proteins, enzymes, RNA, and DNA have been developed or refined. With further refinement, these can be used to increase the reliabilities and frequencies of somatic embryo initiation, development, and conversion to seedlings.

PLANNED ACTIVITY FOR THE PERIOD:

Despite the advances noted above, knowledge of the mechanisms limiting various cloning methods, especially in tissues derived from trees mature enough to have been proven genetically superior, remains fragmentary. As a result, methods

for increasing the effectiveness and efficiency of cloning processes are not available. This new project will concentrate available personnel on developing such methods.

ACTIVITIES OVER THE NEAR-TERM INCLUDE:

Continue using biochemical markers and analyses to characterize the embryogenic potential of cultures and facilitate initiation of embryogenic callus. Accumulate baseline data on development of zygotic embryos, and use results to identify and manipulate factors limiting somatic embryo development and conversion to seedlings.

Use related student research to leverage staff efforts, as numbers and interest permit.

POTENTIAL FUTURE ACTIVITIES:

Document differences between mature and immature tissues, and devise means for rendering mature tissues more easily manipulated in culture. Refine and apply methods for certifying genetic fidelity of seedlings derived from cloning of softwoods and hardwoods. Refine and develop promising new techniques and analyses. Explore techniques for early selection and testing.

COOPERATIVE INVESTIGATIONS

1. North Carolina State University - Cooperative evaluation with Dr. Ralph Mott and Dr. Henry Amerson of procedures for initiating embryogenic cultures of loblolly pine, Norway spruce, and white spruce.
2. Williams College/Merrell-Dow Pharmaceutical Co. - Cooperative study with Dr. Robert Slocum (Williams) plus Drs. A. Bitonti and P. McCann (Merrell-Dow) of polyamine metabolism, and joint preparation of resultant manuscript.
3. International Forest Seed Company - Supply of "rejuvenated" loblolly pine material by Dr. S. Foster for experiments on initiation of embryogenic callus from mature explants.
4. University of Cincinnati - Joint assay with Dr. J. Caruso of endogenous hormone levels, principally ABA and IAA, in embryogenic and nonembryogenic calli. Arrangements now being finalized.

RELATED STUDENT RESEARCH

COMPLETED IN 1987

Daniel Bunker - M.S., Independent Study, entitled "Change in the structure of loblolly pine latewood during delignification." Advisor was T. E. Conners.

Tyrone Cornbower - M.S., Independent Study, entitled "Response of white spruce to mechanical pulping following hemicellulose hydrolysis." Advisors were T. J. McDonough and M. A. Johnson.

Luke Nealey - Ph.D. Program, Organic chemistry orientation, entitled "Isolation and characterization of xyloglucan from suspension cultured loblolly pine cell medium." Advisors were N. S. Thompson and M. A. Johnson.

IN PROGRESS

Michael Bogenschutz - M.S., Independent Study, entitled "Electroporation-mediated genetic transformation of Norway spruce cells." Advisor is M. Becwar (R. J. Dinus).

Daniel T. Bunker - Ph.D. Program, entitled "An investigation of the role of drying strategy in the structure of coatings." Advisor is T. E. Conners.

Lisa G. Dudek - M.S., Independent Study, entitled "Preliminary experiments on encapsulation of zygotic and somatic embryos of Norway spruce." Advisor is N. Rangaswamy.

Patricia Exarhos - M.S., Independent Study, entitled "Electron microscopy study of ultrastructure of Picea abies plants obtained via somatic embryogenesis." Advisor is T. E. Conners.

Russell Feirer - Ph.D. Program, Biochemical orientation, involving biochemical and molecular studies of plant development. In cooperation with the University of Wisconsin, Madison. Advisor is P. Simon.

Scott Fruhwirth - M.S., Independent Study, entitled "A comparative study of organosolv-pulped tension wood and normal wood fibers in the papermaking process." Advisor is T. E. Conners.

Gebran Hammam - M.S., Independent Study, entitled "Comparison of somatic and zygotic cell embryos: Picea abies (Norway spruce) and Pinus taeda (loblolly pine). Advisor is T. E. Conners.

Frederick Lang - M.S., Independent Study, entitled "Construction of a partial genomic library for restriction fragment length polymorphism analysis in sweetgum. Advisor is R. J. Dinus.

Lorrain Logsdon - M.S., Independent Study, entitled "Patterns of and changes in gene expression associated with maturing and germinating seed." Advisor is R. J. Dinus.

Mary Kay Lynde-Maas - M.S. Independent Study, entitled, "Fructose utilization by embryogenic and nonembryogenic suspension cultures of Norway spruce." Advisor is M. A. Johnson.

Thomas J. Merchant - M.S., Independent Study, entitled "The effect of mycorrhizal associations on somatic/zygotic embryo cultures of Norway and white spruce." Advisor is N. Rangaswamy.

Jong-Moon Park - Special Student, Exploratory Research, entitled, "Improving the properties of recycled fibers by chemical and enzymatic treatments." Advisor is M. A. Johnson.

Colleen Walker - M.S. Independent Study, entitled "Optimization and quantification of somatic embryogenic cultures of several conifer species in bioreactors. Advisor is M. Becwar (R. J. Dinus).

INITIATION & MAINTENANCE OF EMBRYOGENIC CALLUS

MICHAEL R. BECWAR

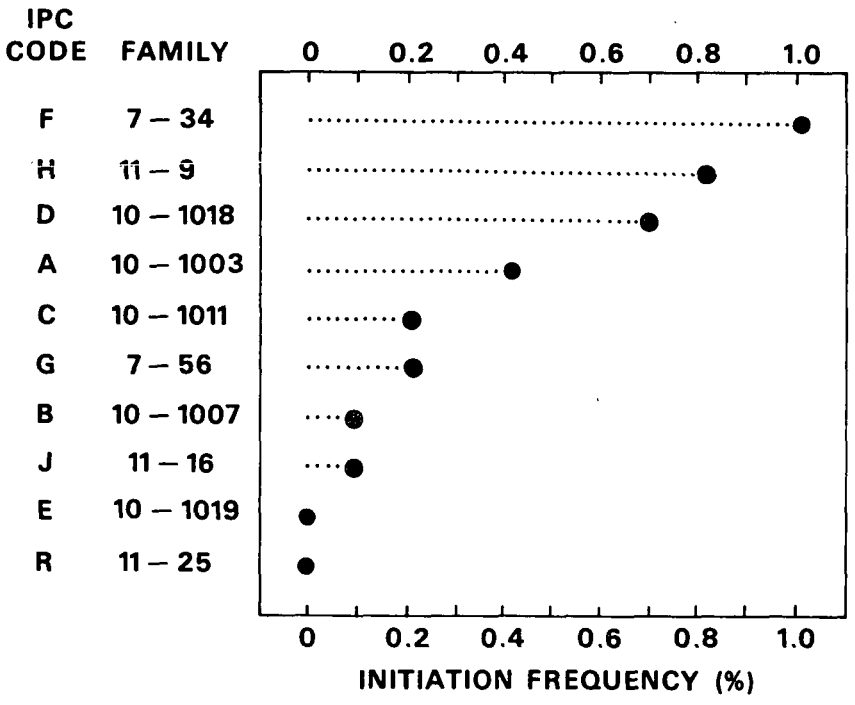
INITIATION AND MAINTENANCE OF EMBRYOGENIC CALLUS IN LOBLOLLY PINE

TOPICS

1. PROGRESS WITH EC LINES INITIATED SUMMER 87
2. STATUS OF INITIATION WITH BRAZILIAN 88 CONES
3. *IN VIVO* AND *IN VITRO* EMBRYOGENESIS IN SPRUCE AND PINE

SUMMARY OF EMBRYOGENIC CALLUS LINES OF LOBLOLLY PINE
INITIATED IN SUMMER 87 AND MAINTAINED TO MARCH 88

Clone		Explants	Embryogenic callus lines	
IPC code	ID no.	cultured	initiated	maintained
A	10-1003	950	4	1
B	10-1007	960	1	0
C	10-1011	965	2	1
D	10-1018	995	7	6
E	10-1019	980	0	-
F	7-34	3311	14	11
G	7-56	1357	4	3
H	11-9	3482	16	16
J	11-16	1392	1	1
R	11-25	1375	0	-
	totals:	15,767	49	39



Comparison of initiation frequency of embryogenic callus among ten families of loblolly pine. Each datum point includes initiation on three medium; MSG O/O, MSCG 5/O, and DCR 3/.5. A total of ≈1000 explants cultured per family.

LIST OF EMBRYOGENIC CALLUS LINES OF LOBLOLLY PINE
INITIATED IN SUMMER 87 AND MAINTAINED TO MARCH 88

Clone	Res. Plan & Line no.	Explant[a]	Initiation Medium	Somatic Embryos Formed[b]
H	571 (LP2H)1	O	MSG 0/0	-
	574 (LP2H)1	O	MSCG 5/0	-
	574 (LP2H)2	O	MSCG 5/0	-
	574 (LP2H)3	O	MSCG 5/0	++
	574 (LP2H)4	O	MSCG 5/0	++
	571 (LP3H)1	O	MSG 0/0	-
	574 (LP3H)1	O	MSCG 5/0	-
	574 (LP3H)2	O	MSCG 5/0	-
	574 (LP4H)1	O	MSCG 5/0	-
	574 (LP4H)2	O	MSCG 5/0	-
	574 (LP5H)1	O	MSCG 5/0	-
	575 (LP6H)1	E	DCR 3/.5	-
	575 (LP7H)1	E	DCR 3/.5	+
	575 (LP7H)2	E	DCR 3/.5	-
	575 (LP7H)3	E	DCR 3/.5	-
	575 (LP8H)1	E	DCR 3/.5	-
F	571 (LP5F)1	O	MSG 0/0	+
	575 (LP6F)1	E	DCR 3/.5	-
	575 (LP6F)2	E	DCR 3/.5	++
	575 (LP6F)3	E	DCR 3/.5	-
	575 (LP6F)4	E	DCR 3/.5	+
	575 (LP7F)1	E	DCR 3/.5	++
	575 (LP7F)2	E	DCR 3/.5	-
	575 (LP7F)3	E	DCR 3/.5	-
	575 (LP7F)4	E	DCR 3/.5	++
	575 (LP8F)1	E	DCR 3/.5	++
	575 (LP9F)1	E	DCR 3/.5	-
D	574 (LP3D)1	O	MSG 0/0	++
	575 (LP3D)2	E	DCR 3/.5	-
	575 (LP3D)3	E	DCR 3/.5	++
	575 (LP3D)4	E	DCR 3/.5	++
	575 (LP4D)1	E	DCR 3/.5	-
	575 (LP4D)2	E	DCR 3/.5	-
G	574 (LP2G)1	O	MSCG 5/0	-
	575 (LP7G)1	E	DCR 3/.5	-
	575 (LP7G)2	E	DCR 3/.5	+
A	574 (LP2A)1	O	MSCG 5/0	++
C	575 (LP3C)1	E	DCR 3/.5	++[c]
J	575 (LP8J)1	E	DCR 3/.5	-

[a] O = ovule explant containing developing immature embryo.
E = immature embryo explant.

[b] Somatic embryos formed when callus transferred to development medium, unless noted. ++ lines showed highest embryogenic potential.

[c] Somatic embryos formed prolifically while still on maintenance medium.

INITIATION EXPERIMENTS WITH BRAZILIAN CONES

DEVELOPMENTAL STAGE OF LOBLOLLY PINE EMBRYOS ISOLATED FROM BRAZILIAN CONES

Collection		Mean embryo size, mm (% cotyledonary)[a]		
		Clone		
no.	date	S	T	U
1	Jan 18	------- proembryo (0) -------		
2	Jan 25	1.3 (50)	0.5 (8)	0.5 (0)
3	Feb 1	1.6 (83)	1.2 (83)	1.7 (83)
4	Feb 8	------- mature (100) --------		

[a] Mean size of 12 embryos randomly sampled from seeds pooled from at least 3 cones. Size not measured on Jan 18 proembryos and Feb 8 fully developed embryos.

COMPONENTS OF BASAL MEDIUM USED FOR INITIATION AND MAINTENANCE IN LOBLOLLY PINE

INORGANICS Macro's (mM)	MS	MSCG	DCR	DZ	MDZ
N (NO_3)	40	1.0	13	53	26
N (NH_4)	21	0	5	6	3
K	20	11	5	47	24
Mg	1.5	ms	ms	.5ms	.5ms
P	1.3	"	"	"	"
Ca	3.0	"	"	"	"
S	1.7	"	"	"	"
Cl	6.0	16.0	1.2	"	"
Micro's (µM)					
Na	202	ms	ms	.5ms	.5ms
Fe	100	"	"	"	"
Mn	100	"	"	"	"
B	100	"	"	"	"
Zn	30	"	"	"	"
I	5.0	"	"	"	"
Mo	1.0	"	"	"	"
Cu	0.1	"	"	"	"
Co	0.1	"	"	"	"
Ni	0	"	0.1	"	"
ORGANICS (mg/L)					
Vitamins					
Nicotinic acid	0.5	ms	ms	ms	.5ms
Pyridoxine	0.1	"	0.5	"	"
Thiamine HCl	0.1	"	1.0	1.0	1.0
Supplements					
Inositol	100	ms	500	1000	1000
Cas. Hydrol.	0	1000	500	500	500
L-glutamine	0	500	250	450	450
glycine	0	ms	2	1	1
Sucrose	30,000	ms	ms	ms	ms

LP OVULE CULTURE: MEDIA TESTED

IPC	NCS
MSCG 5/0	MSG 0/0
MSCG 5/2.5	.5 MSG 1/.5
MSCG 2c/.2	MSCG 1/.5
MDZ 11/5,4	.5 MSCG 1/.5
	DZ 11/5,4
	MDZ 11/5,4

c = chloramben

PERCENTAGE OF RESPONSIVE LP OVULE EXPLANTS CULTURED ON FOUR MEDIA TREATMENTS

Medium	Collection no.	Extruded suspensor tissue (% explants)[a] Clone S	Clone T	Clone U
MSCG 5/0	1	6	1	1
	2	0	7	1
	3	5	11	1
MSCG 5/2.5	1	0	1	0
	2	0	9	1
	3	3	13	0
MSCG 2c/.2	1	0	3	2
	2	7	9	1
	3	3	10	0
MDZ 11/4,5	1	0	0	0
	2	0	0	0
	3	3	1	0

[a] 100 ovules cultured per clone and date.

LP EMBRYO CULTURE: MEDIA TESTED

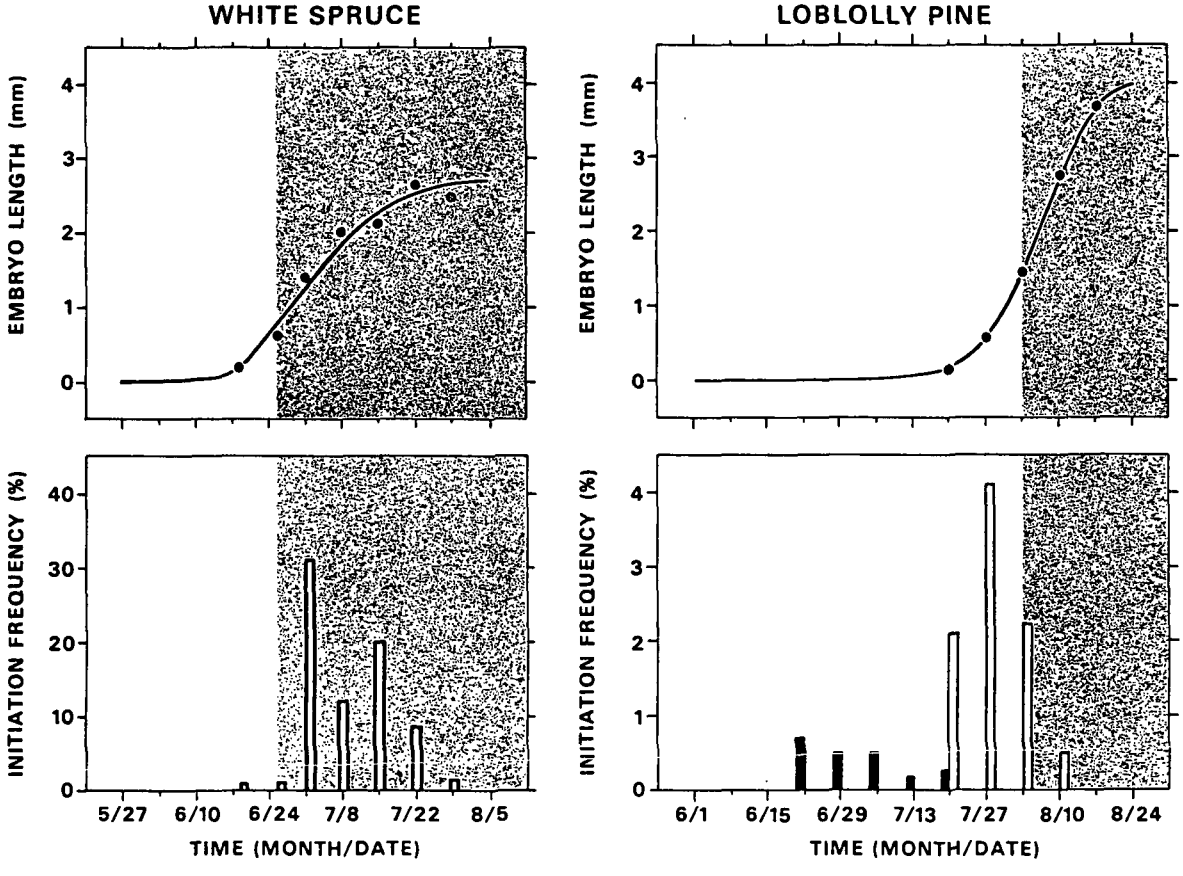
IPC					NCS	
Trt no.	Basal medium[a]		[mM] NH_4	Gln		
1	MS	3/.5	0	0	DCR	3/.5
2	MS	"	0	2	DCR	.5/.25
3	MS	"	1	0	.5 DCR	.5/.25
4	MS	"	1	2		
5	MS	"	5	0		
6	MS	"	5	2		
7	DCR	3/.5	5	2		

a Both DCR and MS media contained the same (DCR) level of NO_3, 13 mM.

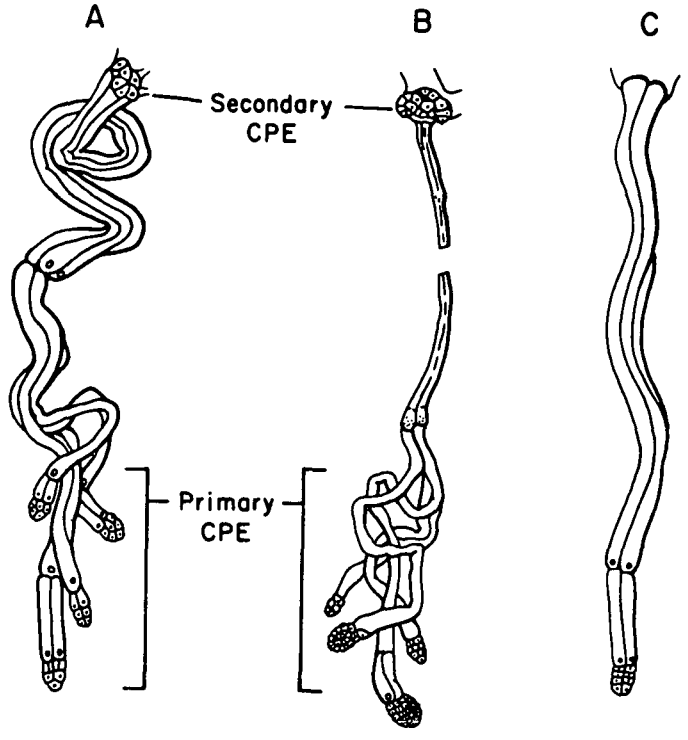
EFFECT OF CHANGES IN THE LEVELS AND FORMS OF REDUCED NITROGEN (NH_4 & GLN) ON DIVISION OF SUSPENSOR CELLS OF LP IMMATURE EMBRYOS

Trt. no.	Basal medium	Conc. (mM) NH_4	Conc. (mM) Gln	Suspensor cell division (% explants)[z] Clone S	Clone T	Clone U
1-6	MS	0,1,5	0,2	3 a	5 a	2 a
7	DCR	5	2	25 b	56 c	20 b

[z] The pooled mean values among treatments 1-6 are given and compared to treatment 7, because there were no significant difference among treatments 1-6. Approximately 60 embryos cultured per treatment.



The relationship of the developmental stage of immature embryo explants (top graphs) to the initiation frequency of embryogenic callus (bottom graphs) in white spruce and loblolly pine. The shaded region denotes ≥ 50% cotyledonary development of embryo explants. Initiation frequencies are averages between two trees of white spruce and two of the most responsive clones of loblolly pine, F (7-34) and H (11-9). Open bars are initiation from immature embryos and solid bars from immature embryos contained in ovules.



Types of *in vivo* polyembryony in gymnosperms. A & B: Cleavage polyembryony occurs in pines. Primary cleavage polyembryony (1°CPE) results from the division of the apical tier cells. Secondary cleavage polyembryony (2°CPE) results from division of suspensor or rosette tier cells. Because these "cleavage embryos" result from mitotic divisions of cells of an individual zygote, they are genotypically identical. C: In spruce cleavage polyembryony does not occur. Multiple embryos can form within a conifer seed due to simple polyembryony (SPE) due to the fertilization of more than one egg per female gametophyte, and the resulting embryos may be genotypically different.

TYPES OF POLYEMBRYONY IN CONIFERS[a]

Genus, common name	SPE	CPE
Pinus, pine	+	+
Picea, spruce	+	-
Pseudotsuga, Douglas-fir	+	-
Larix, larch	+	+/-
Tsuga, hemlock	+	+
Thuja, cedar	+	+
Juniperus, juniper	+	+
Abies, fir	+	+

[a] SPE = simple polyembryony
CPE = cleavage polyembryony

SUMMARY & CONCLUSIONS

1. Eighty percent (39 of 49) of the EC lines of loblolly pine initiated in summer 87 were maintained to March 88.

2. Eleven of the 39 EC lines appear to have high embryogenic potential.

3. EC has been initiated from one (T) of the three clones (S, T, & U) surveyed from Brazil.

4. Of the media tested for ovule culture, MSCG 5/2.5 was best and Gupta and Durzan's was the least effective. Chloramben, at the concentration tested, was not an effective auxin.

5. Suspensor cell division, the origin of EC in pines, was very sensitive to (and significantly reduced by) changes in the forms of media components. This sensitivity demonstrates the plasticity of the process and the potential for increasing EC initiation by making appropriate media modifications.

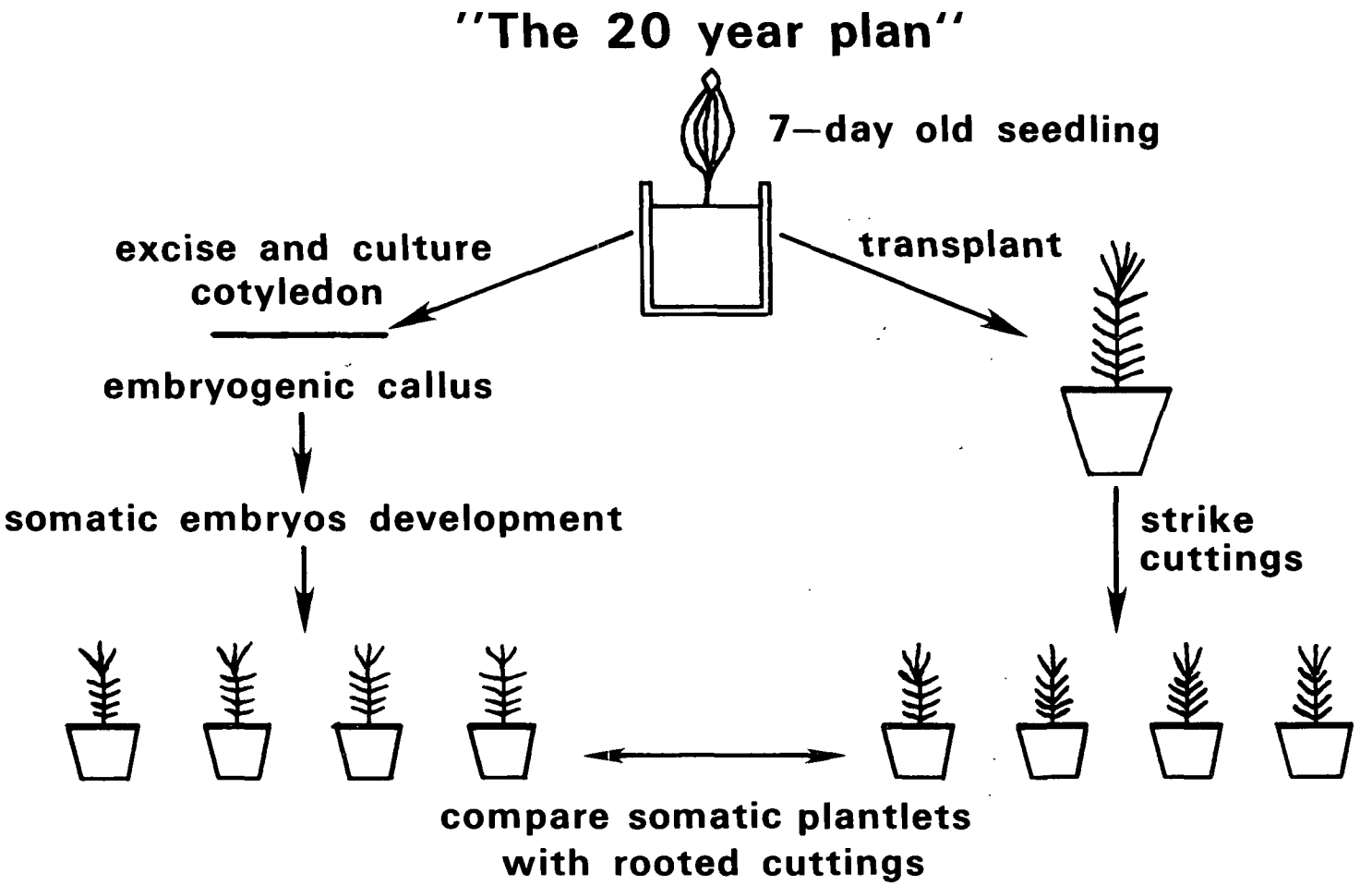
6. Differences in spruce and pine embryogenesis *in vitro*, may relate to inherent differences of their embryogeny *in vivo*; namely, the occurrence of cleavage polyembryony in pines and its absence in spruce. This hypothesis suggests that initiation in Douglas-fir, a species which lacks cleavage polyembryony, will be similar to spruce.

NORWAY SPRUCE, MATURE EXPLANTS
COTYLEDONS, LATEST RESULTS
PROGNOSIS & RECOMMENDATIONS

STEVE WANN

Problem: Initiation of embryogenic callus from embryos sacrifices a significant measure of genetic control.

Solution: Initiate embryogenic callus from embryo/seedling parts and preserve remainder of seedling alive.


"The 20 year plan"
7—day old seedling
excise and culture cotyledon
transplant
embryogenic callus
somatic embryos development
strike cuttings
compare somatic plantlets with rooted cuttings

INITIATION OF EMBRYOGENIC CALLUS FROM 7 - 10 DAY OLD NORWAY SPRUCE COTYLEDONS.

Medium	Photoperiod	n	Frequency, %
MS	dark	50	4
MS	light	50	0
½BLG	light	100	1

All media supplemented with 2,4-D and BA at 2 and 1 mg/L, respectively.

SUMMARY

Although initiation frequencies are low, the entire process (including seedling preservation) is simple and straightforward. This method could prove useful in maintaining genetic control in future studies of embryogenesis in Norway spruce.

EMBRYO DEVELOPMENT & MATURATION
LOBLOLLY PINE, SUMMER COLLECTIONS
RECENT FINDINGS & PROGNOSIS

NAGMANI RANGASWAMY

SOMATIC EMBRYO DEVELOPMENT IN LOBLOLLY PINE

OBJECTIVES

REFINE PROTOCOLS FOR DEVELOPMENT

- FROM PRE-EMBRYOGENIC CELL MASSES (PEMS) TO COTYLEDONARY STAGE
- FROM PRE-COTYLEDONARY (PCE) TO COTYLEDONARY STAGE

EVALUATION OF FACTORS REGULATING DEVELOPMENT

FACTORS REGULATING DEVELOPMENT

EXPLANT: FERTILISED OVULES; EMBRYOS IMMATURE

INITIATION PROTOCOL

CALLUS PHENOTYPE AT INITIATION

TIME ON MAINTAINANCE MEDIUM

LIGHT/DARK

DIFFERENT BASAL MEDIA

GLUTAMINE, SUCROSE (OSMOLARITY) AND ABA

COMPARISON OF SOMATIC WITH ZYGOTIC EMBRYOS AT VARIOUS STAGES OF DEVELOPMENT

EXPLANT GENOTYPE

EXPLANT CHOICE AND INITIATION PROTOCOL

FERTILISED OVULE
(PROEMBRYOS AT EARLY STAGE)
(6/22-7/20)
MSCG 5mg/12,4-D
DARK

IMMATURE EMBRYOS
(7/20-8/20; PCE STAGE)
DCR 3mg/1 2,4-D +
0.5 mg/1 BA.
DARK

CALLUS PHENOTYPE AT INITIATION

LP(2H) 3 & 4
EXTRUDED CALLUS FROM
MICROPYLE OF THE
OVULE
WHITE, MUCILAGINOUS
(PEMS ONLY)

LP(6F) 2
CALLUS FROM ISOLATED
EMBRYOS

WHITE, MUCILAGINOUS;
(VISIBLE SES)
PCES

EXPERIMENT SERIES 1: DEVELOPMENT PROTOCOL FOR LP(2H)3

INITIATION/MAINTENANCE MEDIUM	DEVELOPMENT MEDIUM	NUMBER SES /100 MG TISSUE
MSCG 5/0; MSCG 5/10 (6 WKS)	1/2 HMG + 2.6 ABA + 10 % CW (4 WKS) DCR + 2.6 ABA (2 WKS) 1/2 MSG + 6 % SUCROSE	98
(6 WKS)	DCR + 0.1 BA (6 WKS) DCR + 2.6 ABA (5 WKS)	18
(8 WKS)	MSCG 5/2.5 (4 WKS) 1/2 HMG + 2.6 ABA + 10% CW " " + 1.3 ABA (2 WKS)	191
(8 WKS)	MSCG 5/2.5 (8 WKS) 1/2 HMG + 1.3 ABA (4 WKS) DCR + 2.6 ABA (6 WKS)	140
(8 WKS)	MSCG 5/2.5 (4 WKS) DCR + 2.6 ABA (3 WKS)	150
(10 WKS)	MSCG 5/2.5 BA (4 WKS) MSCG 5/0 BA (4 WKS) 1/2 HMG + 2.6 ABA + 10 % CW (6 WKS)	NONE

EXPERIMENT SERIES 1: DEVELOPMENT PROTOCOL FOR LP(2H)3

INITIATION/MAINTENANCE MEDIUM		DEVELOPMENT MEDIUM	NUMBER SES /100 MG TISSUE
MSCG 5/0	MSCG 5/0 (8 WKS)	DCR + 3 mg/L 2,4-D + 0.5 mg/L BA (4 WKS)	
		1/2 HMG + 2.6 ABA + 10% CW (4 WKS)	96
		" " + 1.3 ABA + 5% CW (2 WKS)	

OTHER TREATMENTS DID NOT PROMOTE DEVELOPMENT FROM PEM STAGE POSSIBLY DUE TO LACK OF CYTOKININ.

ON TRANSFER OF LP(2H)4 LINE TO CYTOKININ CONTAINING MEDIUM (MSCG 5/2.5), PEMS DEVELOPED TO PCES.

EXPERIMENT SERIES 2: EFFECT OF ABA, IBA AND OVULE EXTRACT(O.E).

MEDIUM	LP(2H)3	LP(2H)4
1/4 HMG + 0 ABA	NO VISIBLE PCES	NO VISIBLE PCES
" " + 1.3 ABA	MANY " "	MANY " "
" " + 2.6 ABA	SOME " "	SOME " "
" " + 3.9 ABA	FEW " "	NONE
" " + 10.0 ABA	MANY " "	PCES CALLUSSED OVER
" " + 0.2 IBA + 5.2 ABA	FEW " "	" "
" " + 0.E .10 %	PCES WITH SMOOTHER HEADS	FEW VISIBLE PCES
" " + 0.E .10 % + 1.3 ABA	" " "	PCES WITH WELL FORMED HEADS

LP(6F)2 = NO DEVELOPMENT BEYOND PCE STAGE

CONCLUSIONS

1. WHEN FERTILISED OVULES WERE USED AS EXPLANTS ON MSCG5/O MEDIUM, EXTRUDED CALLUS HAD PEMS.

   WHEN IMMATURE EMBRYOS WERE USED AS EXPLANTS, SOME LINES SHOWED VISIBLE PCES AT INITIATION.

2. LENGTH OF TIME EC LINES ARE ON MAINTENANCE MEDIUM (6-10 WKS), DOES NOT SEEM TO HAVE MUCH EFFECT ON DEVELOPMENT.

3. AT TIME OF INITIAL TRANSFER TO DEVELOPMENT MEDIUM, PRESENCE OF CYTOKININ (0.5 -2.5 mg/l BA) WAS NECESSARY FOR STIMULATION OF DEVELOPMENT FROM PEM STAGE TO PCE STAGE FOR LINE LP(2H) 4.

4. THE RESPONSES OF 2 LINES (LP 2H) 3 & 4 TO ABA AND O.E ARE SLIGHTLY DIFFERENT.

5. LIGHT PROMOTES DEVELOPMENT BETTER THAN DARK.

6. WHITE'S BASAL MEDIUM AND MS BASAL MEDIUM DID NOT PROMOTE DEVELOPMENT

7. ELEVATED LEVELS OF SUCROSE (3-6%) DID NOT AFFECT DEVELOPMENT (WITH OR WITHOUT ABA).

8. SOME SYMPTOMS ASSOCIATED WITH FAILURE TO DEVELOP ARE: PARTIAL OR DELAYED CLEAVAGE OF EMBRYONAL HEADS, TENDENCY TO "CALLUS OVER" AND BROWNING OF THE CALLUS.

BIOCHEMISTRY OF DEVELOPMENT
LIPIDS, ISOZYMES, & REDUCTANTS

MORRIS JOHNSON

LIPIDS, ISOZYMES AND REDOX PARAMETERS

SETTING EXPECTATIONS FOR LP SOMATIC EMBRYO DEVELOPMENT FROM OBSERVATIONS OF LP ZYGOTIC EMBRYO DEVELOPMENT

STAGES OF LP SEED DEVELOPMENT ANALYZED

THE EMBRYO SIZE PROBLEM - ATTEMPTED ANALYSIS BY DIFFERENCE

LIPIDS - Polar lipid peculiar to embryogenic callus is also a feature of LP zygotic embryo development; it may be associated with the embryo in late stages. Some other lipid components show changes also.

PUTATIVE POLAR LIPID PECULIAR TO EMBRYOGENIC CALLI
-DISTRIBUTION AMONG DEVELOPMENTAL STAGES OF LP SEED-

		STAGE			
OVULES	SOURCE	1	2	3	4
	LP S	w	w	wm	wm
	LP T	w	wm	w	w
	LP U	w	w	-	-
GAMETOPHYTES	LP S	w	w	-	-
	LP T	w	w	-	-
	LP U	w	w	-	-

PUTATIVE POLAR LIPID PECULIAR TO EMBRYOGENIC CALLI
-DISTRIBUTION AMONG DEVELOPMENTAL STAGES OF LP SEED-

		STAGE			
OVULES	SOURCE	1	2	3	4
	LP S	w	w	wm	wm
	LP T	w	wm	w	w
	LP U	w	w	-	-
GAMETOPHYTES	LP S	w	w	-	-
	LP T	w	w	-	-
	LP U	w	w	-	-
EMBRYOS		not detectable			

ADDITIONAL OBSERVATIONS ON LIPIDS
IN DEVELOPMENTAL STAGES OF LP SEEDS

1. In all 3 sources, neutral lipids increase with stage.
2. In all 3 sources, a component at R_S= 1.1 to 1.2 increases with stage.
3. In LP S, a component at R_S= 1.8 increases with stage whereas one at R_S= 3.2 appears at stage 4 only.
4. In LP U, a component at R_S= 3.9 decreases and disappears with stage.

PAST OBSERVATIONS ON ISOZYME PATTERN CHANGES IN DEVELOPMENT

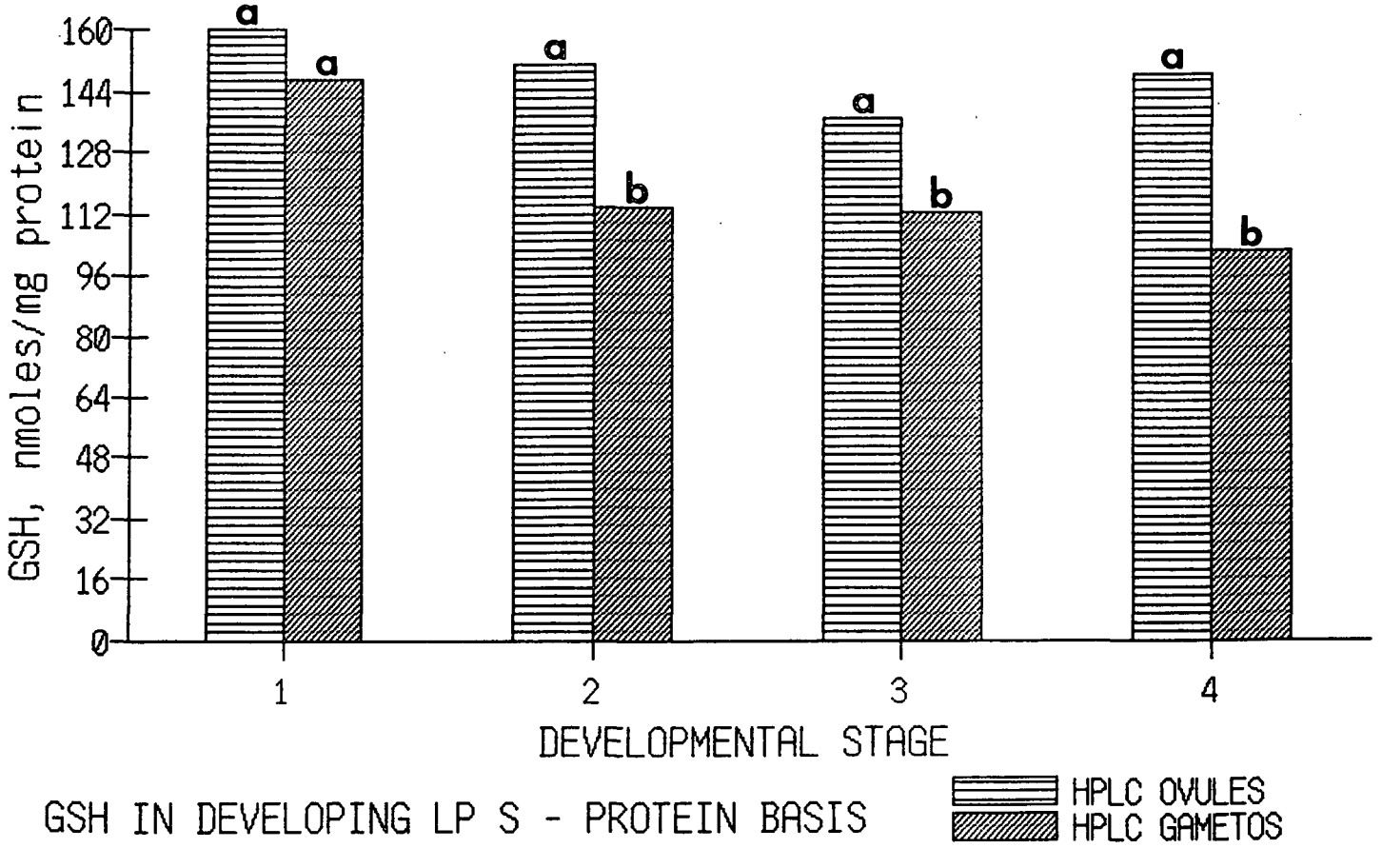
ISOZYME PATTERN CHANGES DURING LP ZYGOTIC EMBRYO DEVELOPMENT

REDOX PARAMETERS: TOTAL REDUCTANTS, GSH, GSSG REDUCTASE

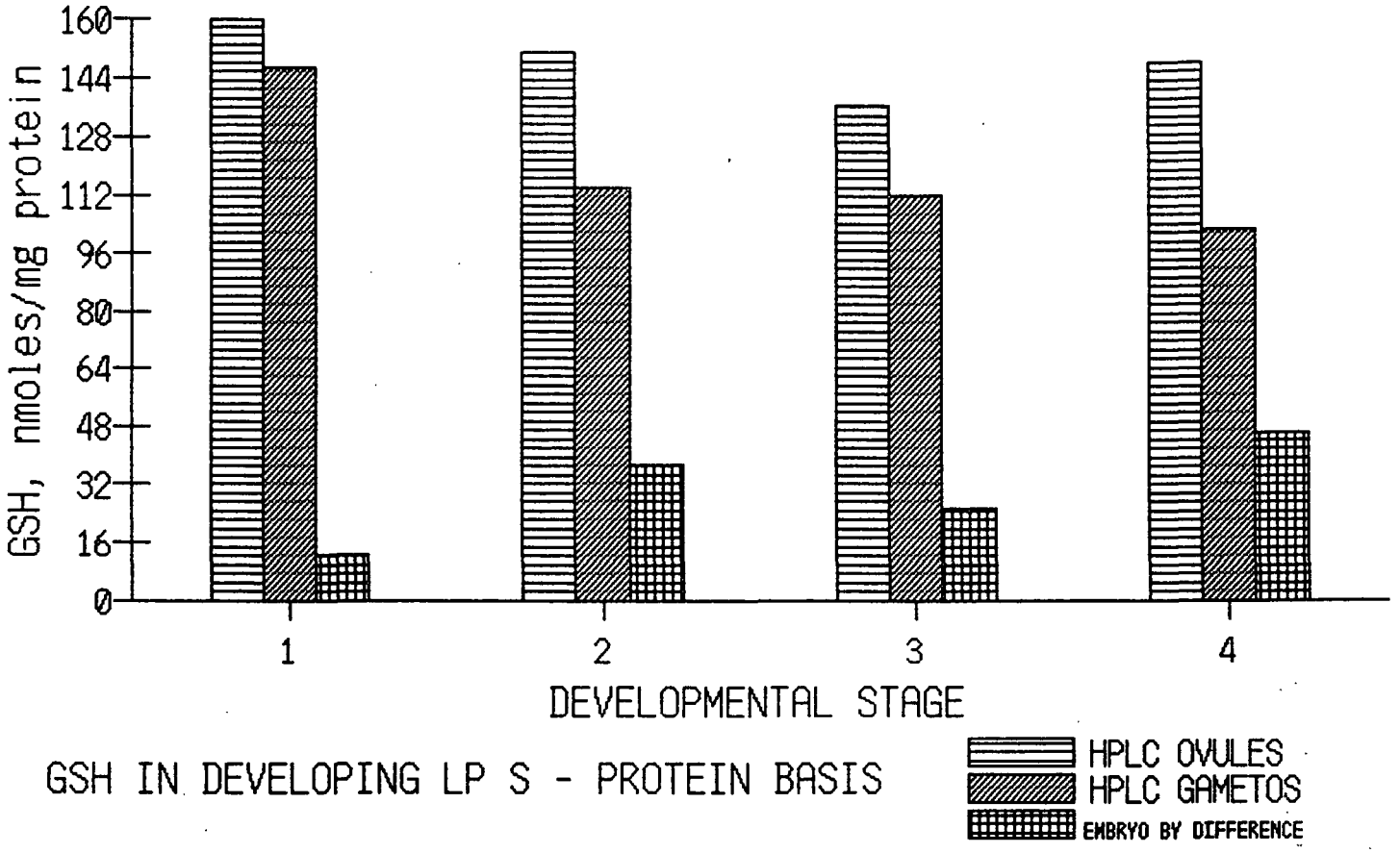
RELATIONSHIP OF PARAMETERS

WHY MEASURE? MECHANISM? INHIBITORS? ISOZYME CONNECTION

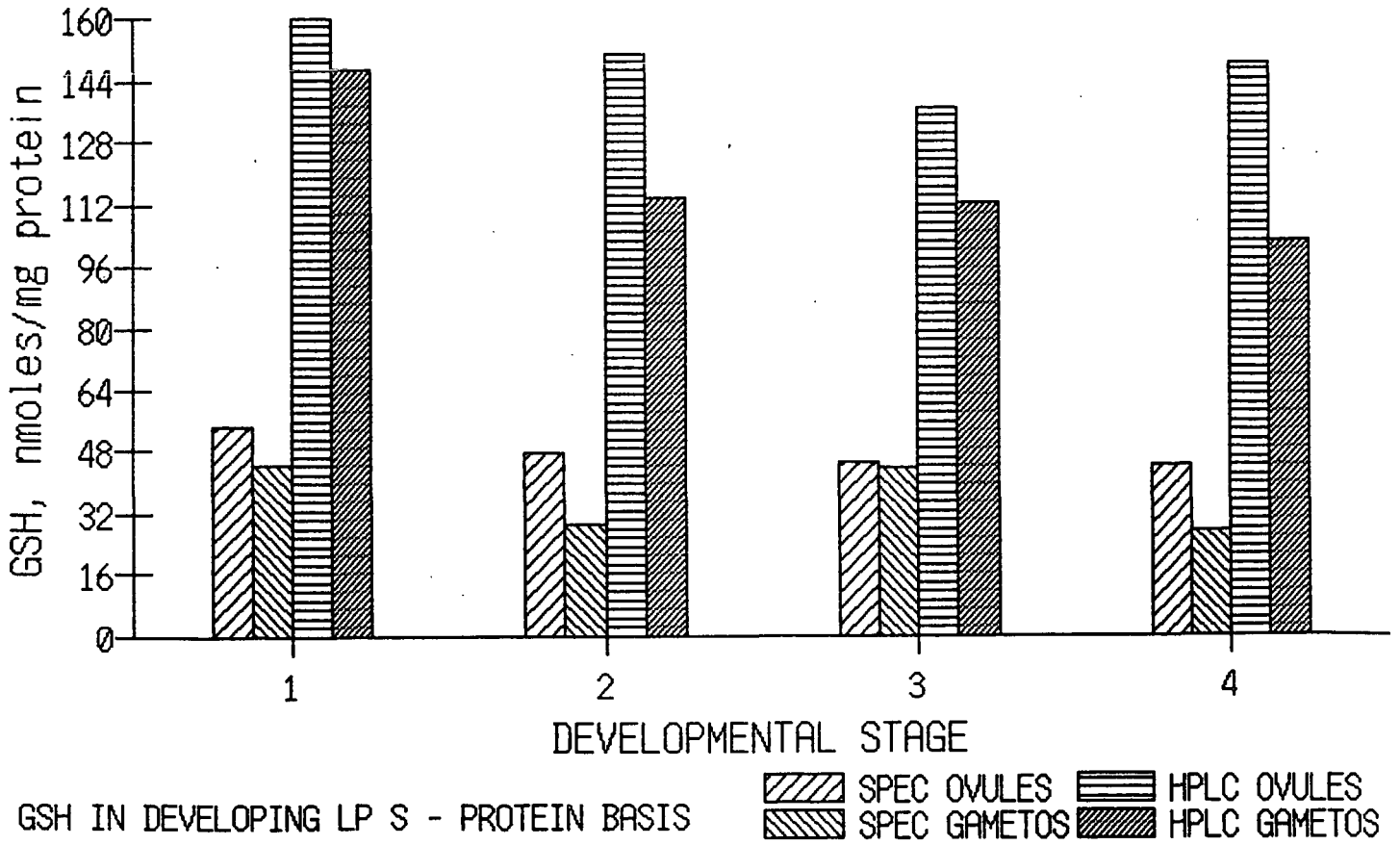
OTHER MECHANISTIC COMPONENTS



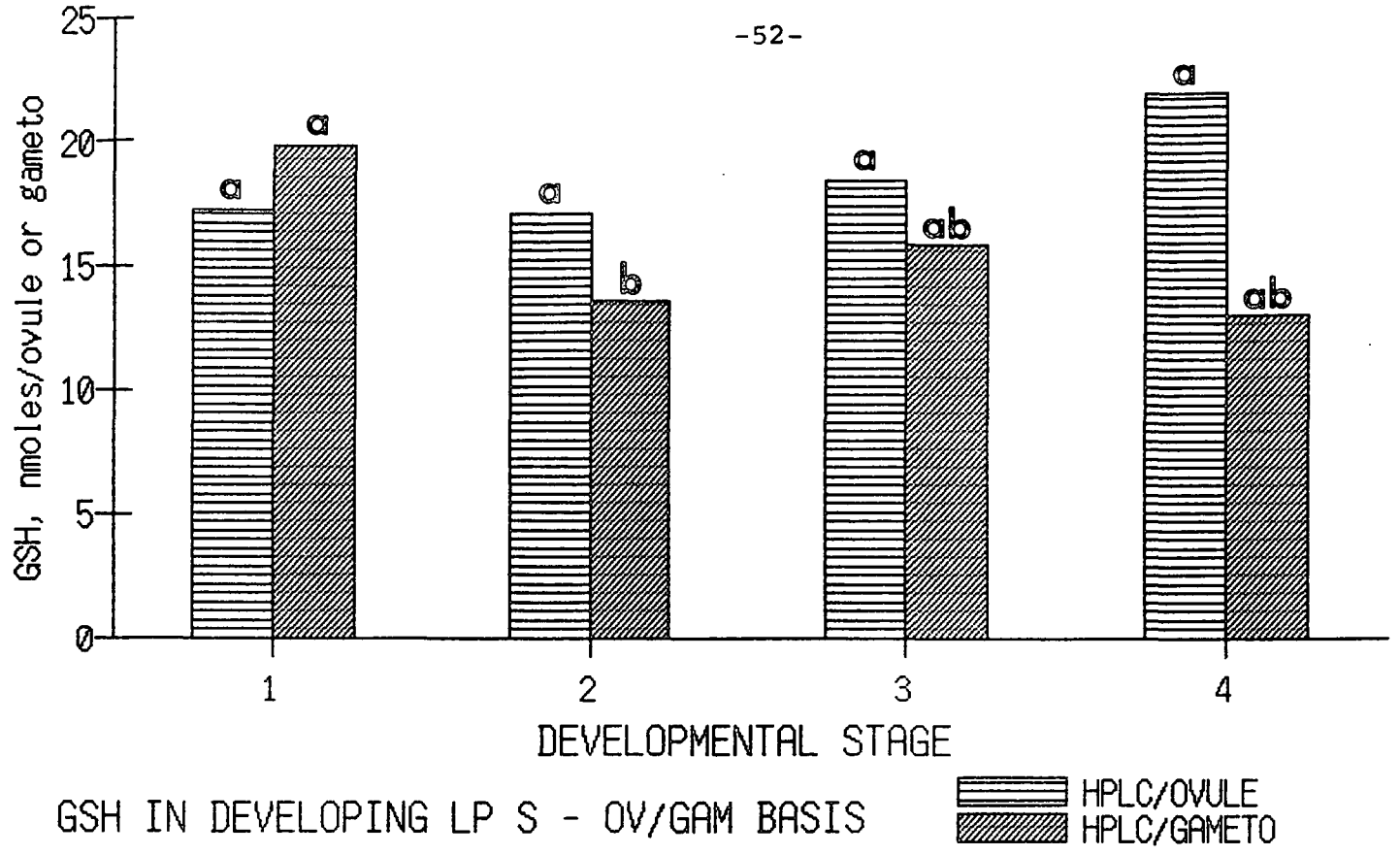
GSH IN DEVELOPING LP S - PROTEIN BASIS



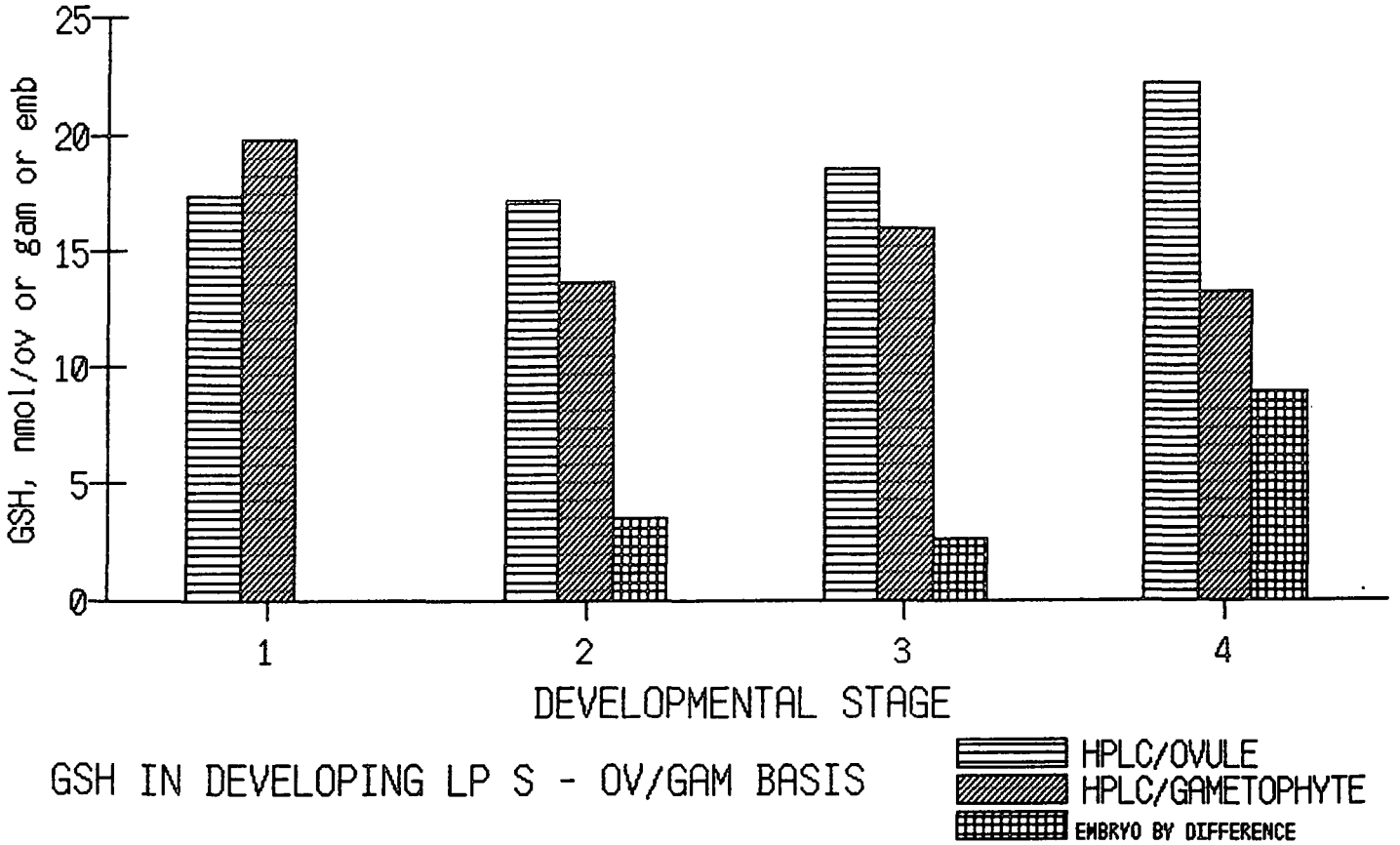
GSH IN DEVELOPING LP S - PROTEIN BASIS



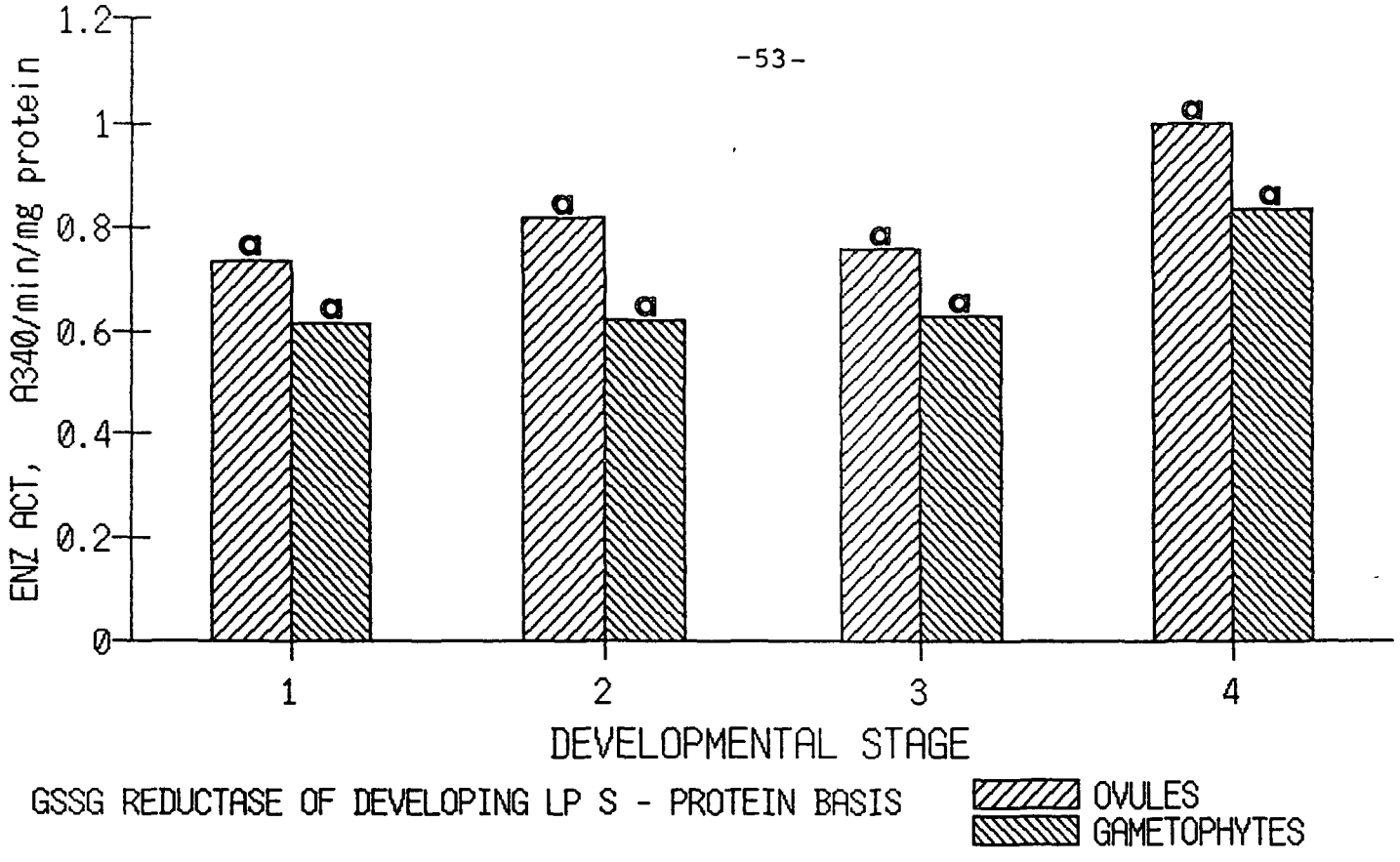
GSH IN DEVELOPING LP S - PROTEIN BASIS



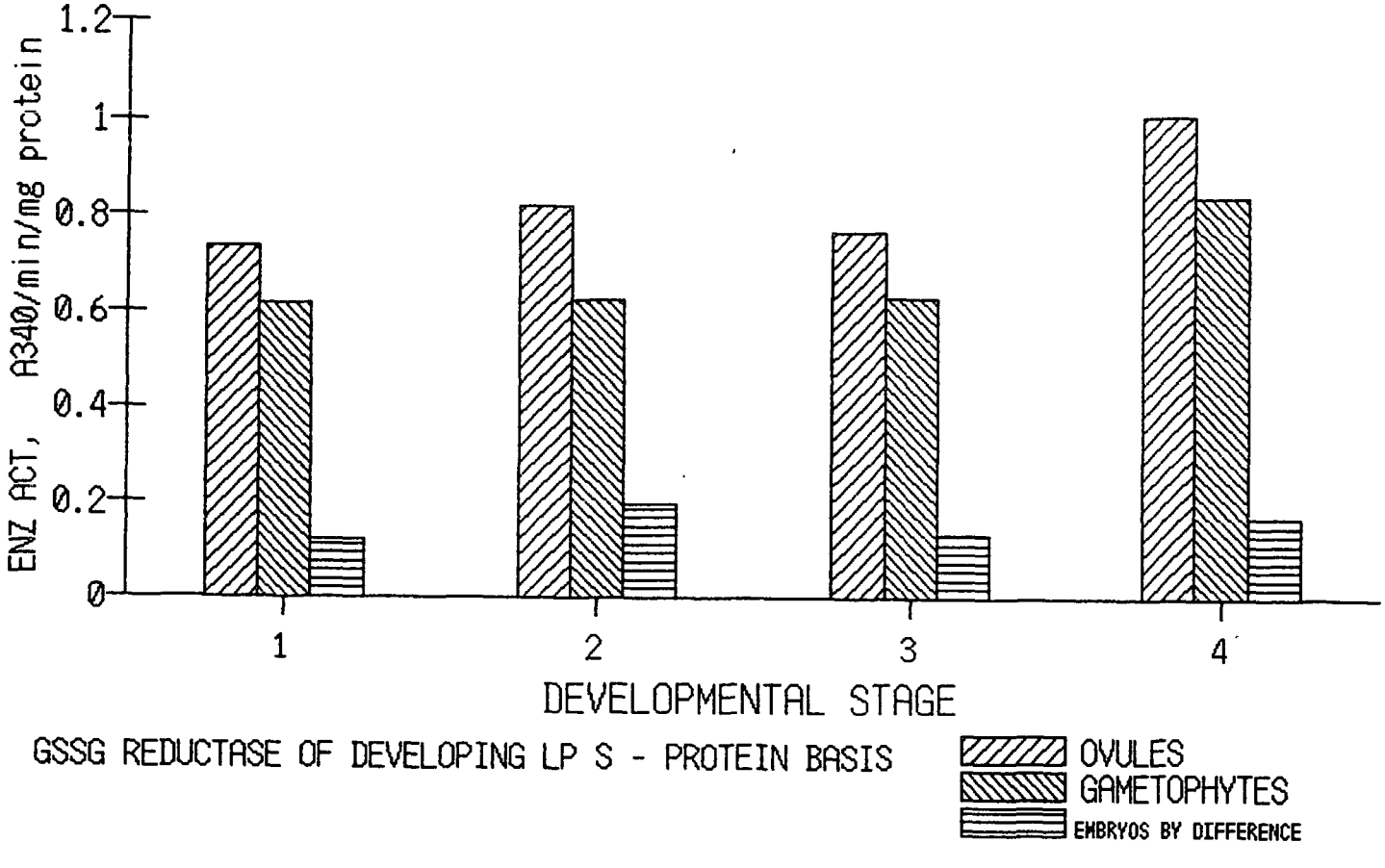
GSH IN DEVELOPING LP S - OV/GAM BASIS



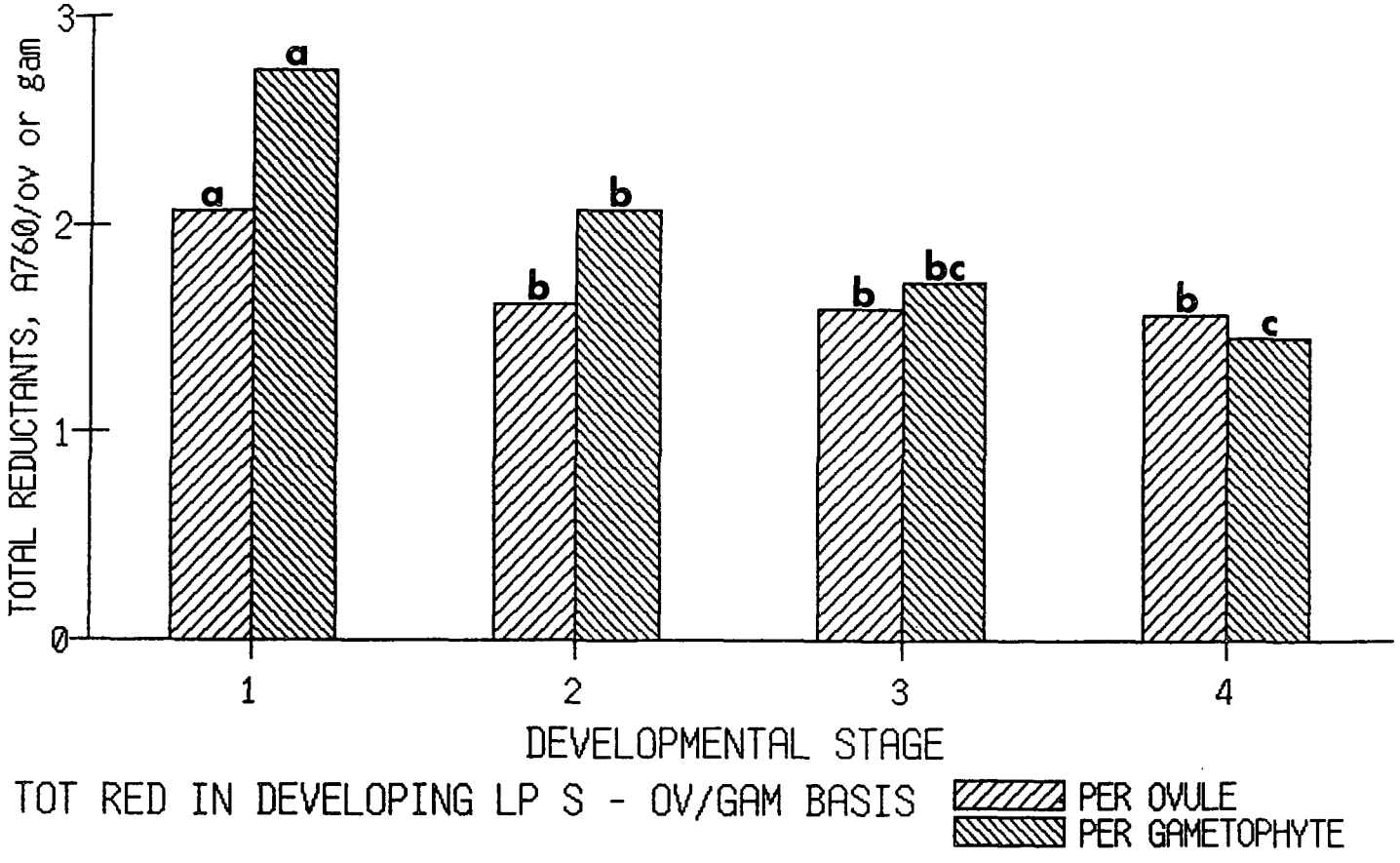
GSH IN DEVELOPING LP S - OV/GAM BASIS



GSSG REDUCTASE OF DEVELOPING LP S - PROTEIN BASIS



GSSG REDUCTASE OF DEVELOPING LP S - PROTEIN BASIS



REDOX PARAMETERS: CURRENT STATUS

BIOCHEMISTRY OF DEVELOPMENT
PROTEIN COMPOSITION

RUSS FEIRER

"Biochemistry of Development: Protein composition and comparisons"

HYPOTHESIS

Synthesis of specific proteins are associated with both natural and in vitro development. These proteins may serve as markers by which the stage of somatic embryo development can be judged.

Previous Results

- **Differences in proteins isolated from embryogenic vs non-embryogenic conifer calli are observed.**
- **Changes in proteins isolated from developing zygotic pine embryos are observed.**

Present Goals

- Compare proteins in somatic embryos (callus) to those observed in developing zygotic embryos.
- Use protein data obtained with developing zygotic pine embryos as a baseline against which to judge the effects of factors (ie growth regulators) that might influence development or conversion of somatic embryos.

The effect of ABA on proteins in excised immature zygotic loblolly pine embryos

The effect of growth regulators on proteins in loblolly pine somatic embryos

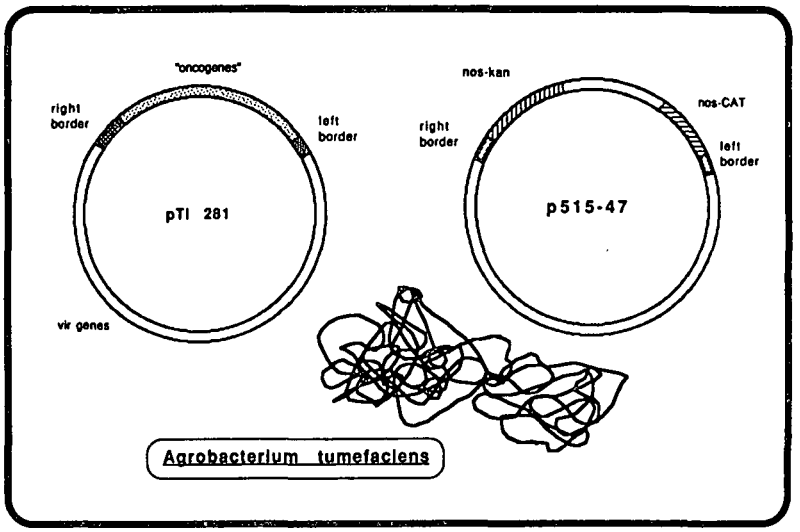
Soluble proteins isolated from zygotic loblolly pine embryos: Effect of genotype

Objective

Can baseline data obtained with zygotic embryos of genotype "A" be compared to data obtained with somatic embryos initiated from genotype "B" ?

Plans

- Continue to compare proteins in developing zygotic embryos to those in somatic embryos. The goal is to judge the effects of factors (ie growth regulators) that might influence development or conversion of the somatic embryos.
- Collect protein data on Norway spruce (model species) zygotic and somatic embryo development.


"oncogenes"
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pTI 281
vir genes
nos-kan
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nos-CAT
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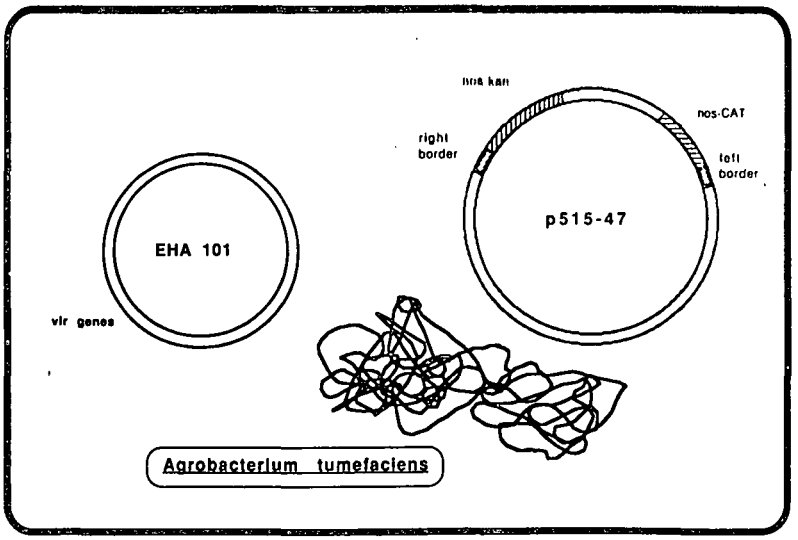
p515-47
Agrobacterium tumefaciens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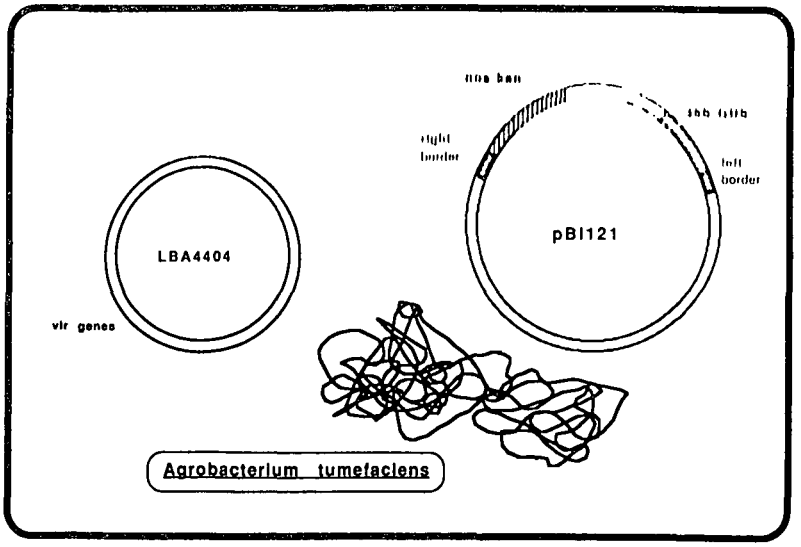
vir genes
Agrobacterium tumefaciens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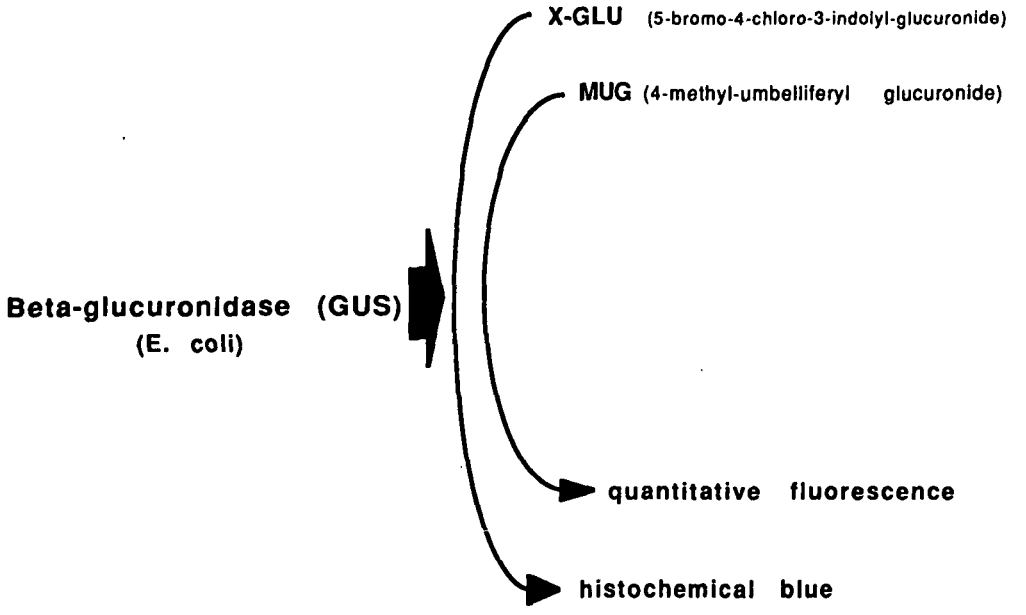

X-GLU (5-bromo-4-chloro-3-indolyl-glucuronide)
MUG (4-methyl-umbelliferyl glucuronide)
Beta-glucuronidase (GUS)
(E. coli)
quantitative fluorescence
histochemical blue

CONVERSION TO SEEDLINGS
ZYGOTIC EMBRYOS, LOBLOLLY PINE

STEVE WANN

ZYGOTIC EMBRYOS USED IN CONVERSION STUDIES

Mature: quick-stratified (30%H_2O_2 for 30 min) and excised dry.

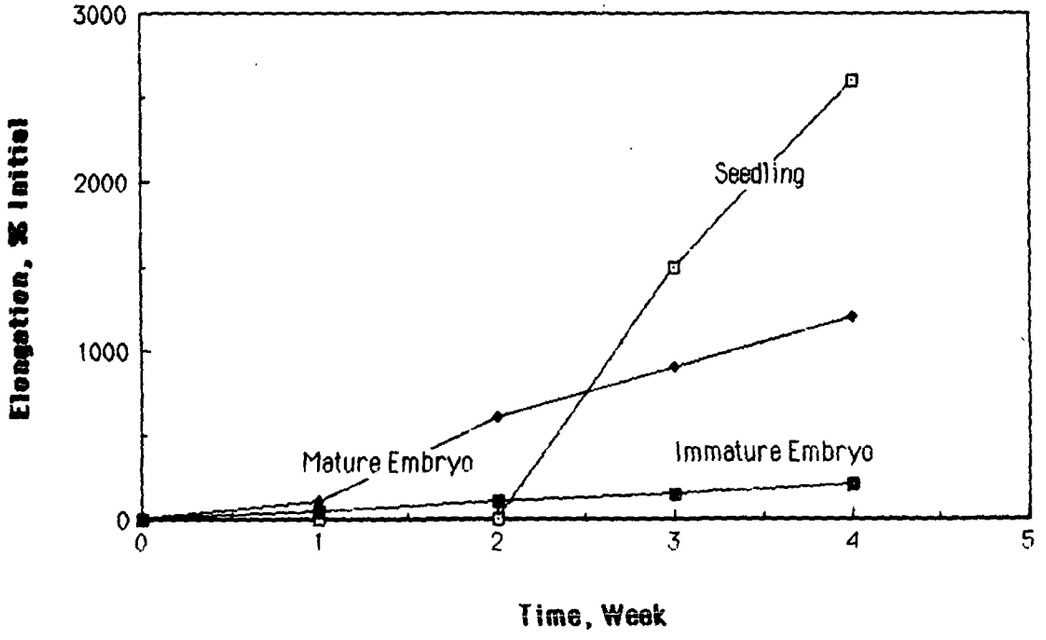
Immature: equivalent to mature embryos in size and apparent development, excised after several weeks of cold storage.

DEVELOPMENT FREQUENCIES FOR COTYLEDONARY ZYGOTIC EMBRYOS

Media	Elongation, %		Root Formation, %	
	Immature	Mature	Immature[a]	Mature[b]
MSG	>95	>95	25	45
½DCR	>95	>95	--	95
WH	>95	--	--	--
½MS	--	--	8	--

[a]Measured after 4 weeks
[b]Measured after 2 weeks

ELONGATION RATES - ZYGOTIC EMBRYOS



CONCLUSION

1. Immature, cotyledonary embryos will undergo development under a wide variety of conditions but a rate very much slower than mature embryos.

2. Root formation and continued germination of both immature and mature embryos is media-dependent.

IMPLICATIONS FOR SOMATIC EMBRYO DEVELOPMENT

1. Somatic embryo conversion will not be as simple as seed germination.

2. Although conversion frequencies are low, rational changes in media composition will likely boost root formation and subsequent survival in soil.

DISCUSSION OF SHORT TERM BENEFITS
CONIFERS & HARDWOODS

STEVE WANN & STAFF

SHORT-TERM BENEFITS, PROJECT 3223

PRELIMINARY REVIEW AND DISCUSSION

DEFINITIONS:

* TECHNIQUES, RESULTS, OR DATA THAT CAN BE APPLIED NOW

* SIMILAR WORK NOT NOW BEING PURSUED BUT THAT COULD BE APPLIED EARLIER THAN SOMATIC EMBRYOGENESIS PER SE

* RESULTS/MATERIAL THAT HAVE ACCELERATED OUR RESEARCH OR THAT OF OTHERS, AND THEREBY SAVED RESEARCH FUNDS

SHORT-TERM BENEFITS, PROJECT 3223

CONIFERS:

* ISOZYME ANALYSES - FIDELITY
  INBREEDING
  EARLY SELECTION

* RESTRICTION FRAGMENT LENGTH POLYMORPHISMS -

  SIMILAR TO BUT MORE DEFINITIVE THAN ISOZYMES

* EMBRYO RESCUE FROM DIFFICULT OR INCOMPATIBLE CROSSES

* "TANDEM" VEGETATIVE PROPAGATION SYSTEMS IN PICEA

* GENE TRANSFER IN PICEA

* FACILITATE/EXPEDITE RESEARCH EXTERNALLY

  SOME EXAMPLES:

  NCSU = EMBRYOGENESIS
  UW, MADISON = CALLUS/PROTOPLASTS
  OARDC = MOLECULAR BASIS OF DEVELOPMENT
  UNIV. OF MAINE = SAME
  UNIV. OF TN = SUSPENSIONS/PROTOPLASTS
  CALGENE PACIFIC = EMBRYOGENESIS
  UNIV. OF GA = HISTOLOGY & HISTOCHEMISTRY

SHORT-TERM BENEFITS, PROJECT 3223

HARDWOODS:

* CLONAL PROPAGATION OF ELITE GENOTYPES NOW

* ISOZYME ANALYSES - FIDELITY
  INBREEDING
  EARLY SELECTION

* RESTRICTION FRAGMENT LENGTH POLYMORPHISMS -

  SIMILAR TO BUT MORE DEFINITIVE THAN ISOZYMES

* "TANDEM" VEGETATIVE PROPAGATION SYSTEMS IN POPULUS AND LIQUIDAMBAR + OTHER SPECIES

* GENE TRANSFER AS ABOVE

* WITH SOME DEVELOPMENT - EARLY TESTING & SELECTION + SOMACLONAL VARIATION

  EXAMPLES = DISEASE RESISTANCE

SUMMARY & DISCUSSION

RON DINUS

PROGRESS: PAC TO PAC

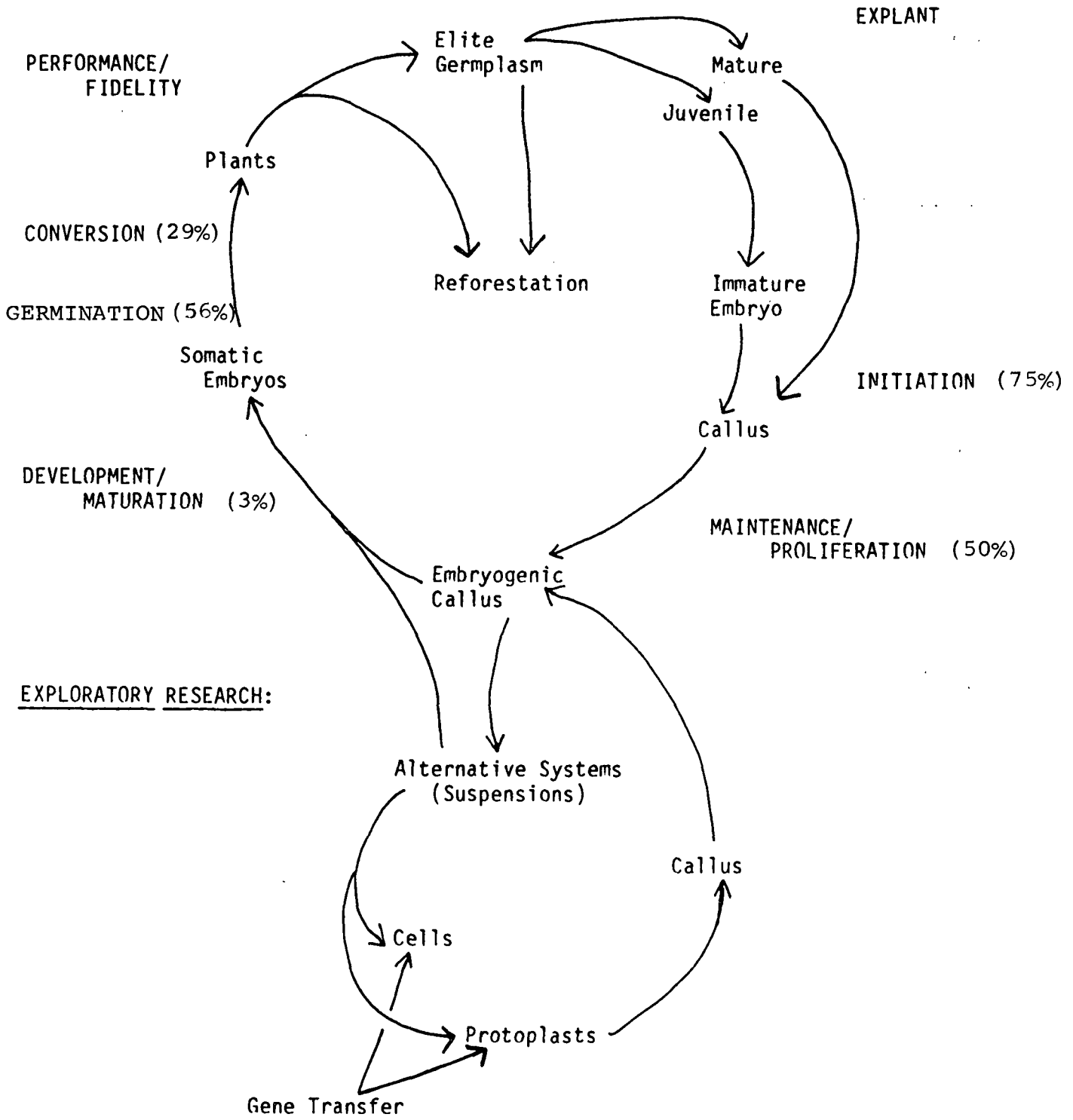
SHORT TERM GOALS	ACCOMPLISHMENTS
REFINE INITIATION PROTOCOLS, TARGET SPECIES	SUMMER LOBLOLLY LINES DOING WELL; HAVE EMBRYOGENIC CALLUS FROM WINTER COLLECTIONS
	DOCUMENTING BEST TREATMENTS & FREQUENCIES. COLLABORATING WITH NCSU
ACCUMULATE BASELINE DATA ON EMBRYO MATURATION, SOMATIC & ZYGOTIC	RESULTS ON SEVERAL FRONTS, PUSHING PROTEINS. ALSO STAINING & SECTIONING. STUDENT PROJECTS
APPLY TOOLS/MARKERS TO IMPROVE EFFICIENCIES OF PROCESS STEPS	CHECKING IMPACT OF ABA ON PROTEIN PATTERNS & DEVELOPMENT. VARIETY OF EXPERIMENTS UNDERWAY
IMPROVE PROTOCOLS FOR DEVELOPMENT & CONVERSION	ABA AIDS DEVELOPMENT; SOME PROMISING MEDIA REFINEMENTS MOVED DEVELOPMENT ALONG. COMPARED ZYGOTIC/SOMATIC DEVELOPMENT PATTERNS
DEVELOP & TEST TOOLS FOR EVALUATING FIDELITY & PERFORMANCE	SLOWED WORK, AWAITING SEEDLINGS. STILL REFINING ISOZYME TECHS., USE FOR VARIETY OF PURPOSES
IMPROVE ALTERNATIVE CULTURE SYSTEMS (SUSPENSIONS)	DEVELOPMENT SLOW, BUT SOMEWHAT BETTER. OBTAINED A FEW N SPRUCE PLANTLETS. STUDENT PROJECT
EVALUATE INITIATION PROTOCOLS FOR MATURE EXPLANTS	LOBLOLLY ROOTED CUTTINGS - CALLUS BUT NOT EMBRYOGENIC, CONTINUING
	N SPRUCE COTYLEDONS - ROUGHLY 1% GIVE EMBRYOGENIC CALLUS

EXECUTE EXPLORATORY RESEARCH	
PROTOPLASTS	LATEST TRIALS = SAME RESULTS; TESTING NEW PROTOCOLS
MOLECULAR TECHNIQUES	CONTINUING, STUDENT INVOLVEMENT, & WESTERN BLOTTING
GENE TRANSFER	SWEETGUM - ADDITIONAL EVIDENCE
	SPRUCE/CARROT - STUDENT WORK ON ELECTROPORATION = -/+
MATERIALS FROM S. HEMISPHERE	SECURED LOBLOLLY, TESTS IN PROGRESS
PUBLICATION/PRESENTATION	CONTINUING TO EMPHASIZE, EXPECTING A BUSY SUMMER

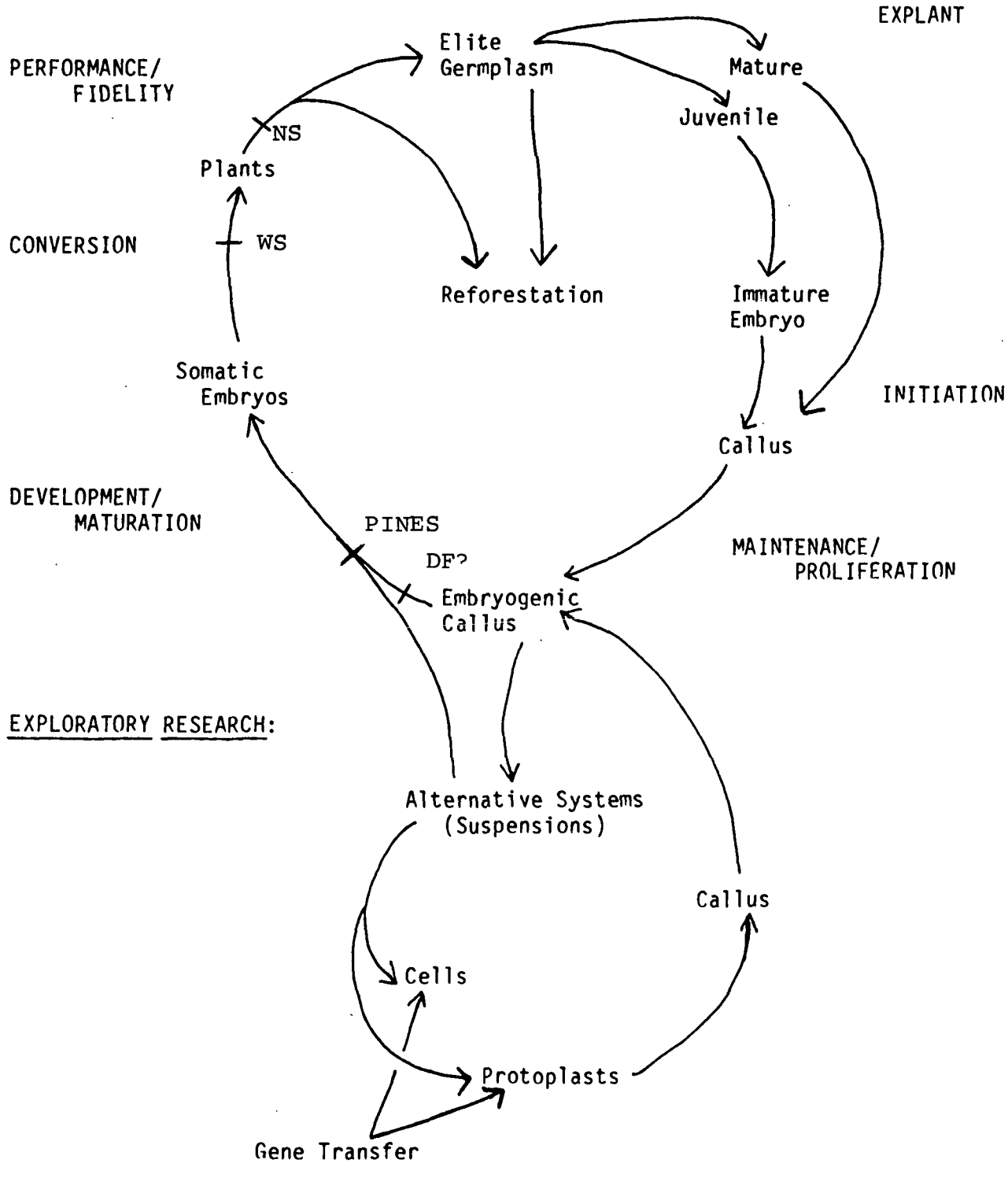
NORWAY SPRUCE STATUS

D/M & C = 0.5%

MASS PROPAGATION OF IMPROVED CONIFERS



MASS PROPAGATION OF IMPROVED CONIFERS



RELATIVE LEVELS OF EFFORT AS INDICATED BY:

RESEARCH PLANS DEVELOPED AND IMPLEMENTED SINCE LAST PAC MEETING FOR EACH RESEARCH AREA AS PERCENTAGE OF TOTAL.

RESEARCH	PLANT MATERIAL			TOTALS
	MODEL SPECIES	TARGET SPECIES	BOTH	
INITIATION	9%	15%	-	24%
DEVELOPMENT/ MATURATION	29	22	4	55
CONVERSION	41	4	-	8
PERFORMANCE/ FIDELITY	-	-	-	-
EXPLORATORY	11	-	2	13
TOTALS	53	41	6	100

TIME SURVEYS = SIMILAR PATTERN

SHORT TERM GOALS

CONTINUE REFINING PROTOCOLS FOR INITIATION IN TARGET SPECIES; RAISE FREQUENCEIES AND RELIABILITIES

ACCUMULATE BASELINE DATA ON HISTOLOGICAL, BIOCHEMICAL, & MOLECULAR EVENTS IN SOMATIC AND ZYGOTIC EMBRYOGENESIS

USE BIOCHEMICAL AND MOLECULAR TOOLS/MARKERS TO IDENTIFY AND TEST TREATMENTS FOR IMPROVING INITIATION AND DEVELOPMENT/MATURATION

DEVISE/REFINE PROTOCOLS FOR DEVELOPMENT/MATURATION & CONVERSION TO SEEDLINGS

IMPROVE PROTOCOLS FOR PROLIFERATION AND DEVELOPMENT IN ALTERNATIVE CULTURE SYSTEMS

CONTINUE WORK ON INITIATION PROTOCOLS FOR MATURE EXPLANTS

EXECUTE EXPLORATORY RESEARCH ON PROTOPLAST CULTURE, PROMISING TECHNIQUES, HARDWOODS, GENE TRANSFER, AND RELATED FRONTS

RECRUIT AND HIRE NEW EMPLOYEES

DEVELOP WHITE PAPER ON HARDWOODS

INTENSIFY EFFORTS TO SECURE GRANTS AND CONTRACTS

PUBLISH/PRESENT PROMPTLY

GLOSSARY

Adventitious - Roots, shoots, embryos, or other organs or tissues developing in an abnormal position.

Agar - Polysaccharide complex extracted from algae. Used as gelling agent in tissue culture medium.

Agarose - A gelling agent derived from agar: the neutral (charge) fraction of agar.

Agrobacterium tumefaciens - Bacterial plant pathogen responsible for crown gall in plants. Harbors a tumor inducing (Ti) plasmid which can be used to transport a foreign gene into a plant cell.

Antibiotic resistance gene - A gene that codes for a protein, which imparts resistance to an antibiotic that allows cells to live in the presence of the drug that would normally kill them.

Archegonium - The flask-shaped container of the ovum (egg cell) of some gymnosperms. The swollen base (venter) contains the egg cell and is surrounded by the neck, with neck canal cells.

Aseptic culture - Surface sterilization of parental explants, free from pathogens, but not necessarily free of internal symbionts.

Asexual reproduction - Reproduction without fertilization. New individuals may develop from vegetative parts such as tubers, bulbs, or rooted stems, or from sexual parts such as unfertilized eggs or other cells in the ovule.

Auxins - A class of plant growth hormones of diverse makeup which cause cell enlargement, apical dominance, and root initiation.

Bacillus thuringiensis - Bacterium which produces a protein having a strong insecticidal activity. Depending upon the strain of the bacteria, the toxin may exhibit specificity toward Lepidopteran, Dipteran or Coleopteran insect groups.

Bacteriophage - A virus that attacks bacteria; also called a phage.

Base (nucleic acid) - A flat, ring compound that forms part of one of the nucleotide links of a nucleic acid chain. The bases are adenine, thymine, guanine, cytosine and uracil (commonly abbreviated A, T, G, C, U).

Base pair - Two bases, one in each strand of a double stranded DNA molecule, which are attracted to each other by weak chemical interactions. Only certain combinations of bases will pair: A-T, G-C and A-U.

Callus culture - Proliferation from a parental explant of many cells in protoplasmic continuity, but having no equivalence with any normal tissue. Same as tissue culture.

Cell differentiation - Internal chemical or ultrastructural changes preceding or accompanying specialization of function.

Cell suspension - Culture of single cells in moving liquid medium, often used to describe suspension cultures of cells and cell aggregates.

Chloroplast - A membrane-enclosed subcellular organelle containing chlorophyll. Chloroplasts are the sites of photosynthesis. They contain DNA and ribosomes and can replicate.

Clonal propagation - Propagation of a group of plants derived from a single individual (ortet) by asexual reproduction. All members (ramets) of a clone have the same genotype and consequently tend to be uniform.

Clone - 1. (verb) to undergo the process of creating a group of identical DNA molecules or genes derived from a single source. 2. (noun) a group of genetically identical cells (plants), all derived from a single ancestor.

Cloning vector - Small plasmid, phage or virus DNA molecules used to transfer a DNA fragment or gene from a test tube to a living cell. Some vectors are capable of multiplying inside living cells (bacteria) to result in the multiplication or cloning of the transferred DNA or gene.

Codon - A group of three nucleotides coding for an amino acid.

Conversion or Transfer to Soil - Survival and continued growth of an <u>in vitro</u> derived plantlet (germinant) in soil (nonaxenic conditions).

Coumarins - A class of phenylpropanoid phenolic compounds of which coumarin itself typifies the structures.

Cotyledon - The leaf formed directly from the embryo of an angiosperm or gymnosperm. There may be one (in monocotyledons), two (in dicotyledons), or several (in gymnosperms). They act as storage organs in nonendospermous seeds and as the first photosynthetic organs in endospermous seeds.

Cytokinins - A class of plant growth hormones associated with cell division, assisting with the transmission of the genetic information from the genes to the proteins.

cDNA (complementary DNA) - DNA synthesized from an RNA template in test tubes using the enzyme reverse transcriptase. The DNA sequence is thus complementary to that of the RNA. cDNA is usually made with radioactive nucleotides and is used as a hybridization probe to detect specific RNA or DNA molecules (genes).

Denature - In reference to DNA, denaturation means conversion of double stranded to single stranded DNA.

Development - Any or all of the steps subsequent to the first asymmetric cell division that result in the formation of a complete plant.

2D TLC - Two-dimensional thin-layer chromatography.

Diploid - Having two sets of chromosomes in the nucleus. One-half of the chromosomes are contributed by one parent, one-half by the other parent. Many higher organisms are diploid except for their sex cells and associated tissue.

Electroinjection - Method of transporting naked DNA into a plant cell having a cell wall using a short duration DC electrical pulse (see electroporation).

Electroporation - Method of transporting naked DNA (gene) into a protoplast using a short duration DC electrical pulse.

E. coli (Escherichia coli) - A bacterium commonly found in the digestive tracts of many mammals, including humans.

EM - Electron microscope.

Embryo - The young plant developing in the megagametophyte from the fertilization of an egg cell, or without fertilization. In aseptic cultures, adventitious embryos show polarization followed by the growth of a shoot from one end and a root from the other end.

Embryogenesis - Initiation of embryoids or embryos from cultured cells.

Embryoid - A cell group approximating an embryo, but having a more random cell arrangement.

Enzyme - A protein molecule that catalyzes a specific chemical reaction.

ER - Endoplasmic reticulum. A system of membranes (originating from the external membrane of the nuclear envelope) that permeates the cytoplasm and that may or may not be covered with ribosomes.

Erosion zone - Zone in the gametophytic tissue below the archegonium that is degraded by the developing embryo.

Eucaryotic cells - Cells with true nuclei bounded by nuclear membranes and which undergo meiosis.

Excise - To cut or isolate callus tissue from its parental explant or to remove adventitious shoots from callus tissue for rooting.

Explant - A plant part excised and prepared for aseptic culture by surface sterilization followed by the exposure of live cells to a nutrient medium.

Fertilization - The normal union of two gametes during sexual reproduction.

Fidelity - Preservation of the original genotype and phenotype.

Flavonoids - A class of phenolic compounds usually consisting of two hydroxylated aromatic rings joined by a three-carbon chain.

Gametophytic tissue - Haploid tissue of the seed that surrounds the developing embryo during the latter stages of embryogenesis.

Gel electrophoresis - A method for separating molecules based on their size and/or electrical charge. Molecules are forced to run through a gel (e.g., agarose or polyacrylamide) by placing them in an electric field. The speed at which they move depends on their size and/or charge.

Gene - One of the units of inherited material carried on a chromosome; arranged in a linear fashion and indivisible.

Gene cloning - A way to use microorganisms to produce millions of identical copies of a specific region of DNA or gene.

Gene pool - Reservoir of genetic variability available for use in genetic improvement of tree species.

Genetic engineering - The formation of new combinations of heritable material by the insertion of nucleic acid molecules into a vector system so as to allow their stable incorporation into a host organism in which they do not naturally occur.

Genetic gains - Average improvement in progeny over the mean of the parents.

Genetic variability - The variation existing in a given population (species, for example) with respect to particular genes or arrangement of genes.

Genome - May refer to the full genetic complement in the haploid set of chromosomes of a species, but one may speak of nuclear, chloroplastid and mitochondrial genomes.

Genotype - The genetic makeup of an individual; carried in the chromosomes.

Germination - Production of a germinant (plantlet with primary root) from a mature embryo.

Grana - Association of thylakoids in a stack.

Groundplasm - Homogeneous plasma (matrix) remaining after cell organelles and particles have been excluded.

Haploid - Having the reduced chromosome number, i.e., having one set of chromosomes in the nucleus. This is normal in sex cells, which have only half the number of sets occurring in diploid vegetative cells.

Homologous - Describing regions of DNA molecules that have the same nucleotide sequence. Complementary base pairing can occur between homologous regions in two different DNA molecules.

Hormone - Any growth substance which is generally transported to the site of action and can stimulate growth or cell enlargement (auxins), cell division (cytokinins), stem elongation (gibberellins), or can retard growth as in the abscission of leaves (ethylene).

Hybrid vigor - The increase in vigor, size and fertility of a hybrid as compared with its parents, resulting from the union of genetically different gametes and assumed to be due to special recombinations of dominant and recessive genes (heterosis).

Hybridization - The production of offspring of genetically different parents.

Hypocotyl - The part of a seedling axis between the radicle and the cotyledon(s).

Induction - To cause initiation of a plant structure, organ or process.

Initiation - The formation of callus from an explant.

Inoculation density - "ID" is the volume of cells per unit of medium, i.e., µL/mL.

Inoculum - A small piece of tissue cut from callus, or a small amount of cell material from a suspension culture placed in contact with fresh medium for continued growth of the culture. Inocula (plural).

Interspecific hybrid - The progeny from matings between species.

Intraspecific hybrid - The progeny from matings within species.

Intron - A noncoding section of a gene that is spliced out of mRNA before translation into proteins.

In vitro - Outside the living organism.

In vivo - Within the living organism.

Isozymes - Multiple forms of a single enzyme.

Kanamycin - Antibiotic that disrupts protein synthesis in some bacteria and plants.

Lamda - The name of a particular bacteriophage (virus) used extensively in gene cloning.

Launch - (Induction), to cause the initiation of a process that will result in the development of a plant structure (shoots, roots, or embryos); sometimes used to describe the log phase of the growth cycle.

Lipids - Any of a group of biochemicals which are variably soluble in organic solvents and barely soluble in water.

Maintenance - The perpetuation of callus by subculture.

Maturation - Development of proembryo to cotyledonary (mature) embryo.

Milieu - The whole chemical and physical environment of a culture.

Meristem - A localized group of cells, actively dividing and undifferentiated but ultimately giving rise to permanent tissue such as shoots, roots, wood or bark.

Meristemoid - A localized group of cells in callus tissue, characterized by an accumulation of starch, RNA and protein, and giving rise to adventitious shoots or roots.

Mitochondria - Small bodies in spaces of the cytoplasm. They are spheres or rods, and are the sites of many important aerobic enzymatic processes. The inner layer of the wall is infolded into fingerlike processes.

Morphogenesis - Initiation of organized tissue in callus or suspension cultures.

mRNA (messenger RNA) - RNA that is used by the ribosome to synthesize proteins.

Nick translation - A procedure for radiolabelling DNA in vitro. Used to make a radioactive probe.

Nuclease - A general term for an enzyme that cuts DNA or RNA.

Nucleic acid - DNA or RNA.

Nucleotide - One of the building blocks of nucleic acids. A nucleotide consists of three parts: a base, a sugar and a phosphate.

Nutrient medium - A solid or liquid combination of major and minor salts, an energy source (sucrose), vitamins, hormones, and occasionally other defined or undefined supplements. Usually made up from previously prepared stock solution, then sterilized by autoclaving or filtering through a micropore filter. Media (plural).

Organized tissue - Tissue composed of regularly differentiated cells.

Organelle - A complex cytoplasmic structure of characteristic morphology and function, such as a mitochondrion or plastid.

Organogenesis - Initiation of roots or shoots from callus meristemoids.

Packed cell volume - "pcv" is the volume of cells determined by centrifugation.

Parasexual hybridization - Hybridization resulting from asexual fusion of cells, either diploid or haploid.

Passage - The duration of growth of callus or cell material from one subculture to another.

Performance - Response of the regenerated somatic plant to the environment relative to the original plant or suitable control plants.

Photoperiod - Length of daily light cycle.

Plasmalemma - The semipermeable unit membrane surrounding and containing the cell cytoplasm. In plant cells, it is pressed up against the inner surface of the cell wall.

Plasmid - A small circular DNA molecule found inside bacterial cells. Plasmids reproduce every time the bacterial cell reproduces. Once infected, the bacteria will always contain a plasmid. Some plasmids continue to replicate in a bacterial cell so that a single cell may contain 200 plasmids. Plasmids are thus used to clone a gene.

Polyploidy - Having three or more times the haploid number of chromosomes.

Procaryotic cells - Single-celled organisms and reproducing entities that lack a membrane-bound nucleus; they do not undergo meiosis; these include the viruses, bacteria, and blue-green algae.

Probe - A radioactive DNA or RNA molecule used to detect the presence of its complementary strand on an electrophoretic "gel" by hybridization and autoradiography.

Proembryo - The very earliest stage of embryo development before suspensor cell elongation occurs.

Proliferation - Increase in mass of callus, cells, somatic proembryos, etc., involving an increase in numbers.

Prolamellar body - Semicrystalline structure from which thylakoid membranes arise during chloroplast development in dark grown seedlings.

Promotor - A short nucleotide sequence on DNA recognized by RNA polymerase to initiate transcription (synthesis of mRNA).

Proplastids - A group of plastids which are progenitors of chloroplasts.

Protoplast - Spherical cell protoplasm (cytoplasm + nucleus) bounded by a membrane but no cell wall.

Protoplast fusion - Union of two protoplasts into one cell.

Recombinant DNA (rDNA) - Chimeric DNA molecule formed by cutting and splicing of DNA (genes).

Recovery - The overall process of development starting with the proembryo. Recovery frequency = maturation frequency x germination frequency x conversion frequency.

Restriction endonucleases - (Restriction enzymes) enzymes that cut DNA at specific nucleotide sequences yielding fragments of various sizes. These enzymes are isolated from a variety of bacteria, and are identified by a three letter abbreviation consisting of the first letter of the genus and the first two letters of the bacterial species name, followed by the strain number (e.g., a particular enzyme isolated from an E. coli strain is designated Eco R1).

RFLPs (restriction fragment length polymorphisms) - DNA molecules from the same gene in two different individuals may differ slightly, and fragments of different length are formed when the gene is digested with a restriction enzyme. Since unequal-sized fragments travel at different speeds in an electrophoresis gel, the two fragments visualized by a radioactively-labeled homologous probe would appear as different bands on the gel. This is a RFLP.

Reverse transcriptase - An enzyme purified from tumor viruses that synthesizes DNA complementary to an RNA template.

Ribosomes - Organelles containing protein and RNA. They are seen as dense particles in electron micrographs. They are found in all types of cells in which protein is being synthesized.

RNA - Ribonucleic acid. RNA is usually single stranded.

RNA polymerase - The enzyme responsible for making RNA complementary to a DNA template. RNA polymerase binds at specific nucleotide sequences (promotors) in front of genes in DNA. It then moves through a gene and makes an RNA molecule that contains the information contained in the gene.

SEM - Scanning electron microscope.

Sequence - The order of the nucleotides in the DNA or RNA chain.

Somatic - Diploid body cells of an organism; those cells other than germ cells.

Somatic cell hybrid - The plant resulting from fusion of protoplasts from somatic cells of genetically different sources.

Splicing - Removal of introns from the "immature" form of eukaryotic mRNA. Carried out in the nucleus of the cell.

Subculture - Dividing agar grown callus or liquid cell suspensions for transfer to fresh medium.

Suspension culture - Cells or cell aggregates dispersed and growing in moving liquid medium.

Suspensor - Elongated, vacuolated cells subtending the embryonal cells in a developing zygotic embryo.

Tannins - A class of complex phenolic compounds known for their astringency and ability to tan the proteins of animal skins. There are two major types of tannins, the hydrolyzable and the condensed tannins.

TEM - Transmission electron microscope.

Template - A pattern of nucleotide sequences in DNA or RNA used by polymerases to specify the sequence in a new polymer by complementarity.

Tetracycline - An antibiotic that kills bacteria by blocking protein synthesis.

Thylakoids - Complex system of flattened membranes within a chloroplast; are often found in stacks to form grana.

Ti plasmid - The plasmid carried by the bacterium Agrobacter tumefaciens which is used to carry foreign genes into a plant cell.

Tissue culture - General term for callus and cell cultures.

Totipotency - A cell characteristic in which the cell retains the potential of forming all the cell types of the adult organism.

Transcription - The process of converting information in DNA into information in RNA. The copying of a gene into RNA. RNA polymerase is the enzyme that executes this conversion of information.

Transformation - The process whereby a cell takes up free DNA such that the free DNA (gene) becomes a permanent part of the cell's genome.

Translation - The process of converting the information in mRNA into protein. Also called protein synthesis.

Transposon - A short section of DNA capable of "jumping" to another region of a chromosome or to a different chromosome.

Transposon tagging - Method of using a transposon to locate a gene. When a transposon inserts into a chromosome, it causes a knockout mutation leading to a distinct mutant phenotype. A radioactive probe made from this transposon can then be used to identify the DNA sequence (gene) into which it had been inserted. The gene can then be localized on a gel and perhaps on a particular chromosome from the mutant plant. In short, the mutated gene is tagged or made identifiable by the transposon.

Ultrastructural - Sublight microscopic, intracellular structure.

Vacuole - A fluid-filled space in a cell. A single vacuole, taking up most of the volume of the cell, present in many plant cells, and containing a cell sap which is isotonic with the protoplasm.

Vegetative cells - Nonreproductive cells such as haploid cells from female gametophytes of conifers or diploid somatic cells.

Vesicle - Small membrane-bound body in the cytoplasm.

Zygote - Fusion product of male and female sex cells or fusion product of protoplasts.

AMINO ACIDS ABBREVIATIONS

ala	alanine
arg	arginine
asn	asparagine
asp	aspartic acid
cit	citrulline
cys	cysteine
γ-aba	aminobutyric acid
gln	glutamine
glu	glutamic acid
gly	glycine
his	histidine
hyp	hydroxyproline
ile	isoleucine
leu	leucine
lys	lysine
met	methionine
orn	ornithine
phe	phenylalanine
pro	proline
ser	serine
thr	threonine
trp	tryptophan
tyr	tyrosine
val	valine

CUMULATIVE LIST OF ABBREVIATIONS

AA	Ascorbic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
ADC	Arginine decarboxylase
ADP	5'-Adenosine diphosphate
AMP	5'-Adenosine monophosphate
ANOVA	Analysis of variance
AOA	Aminooxyacetic acid
AOAA	Aminooxyacetic acid
AOPP	α-Aminooxy-β-phenylpropionic acid
ATP	Adenosine triphosphate
AVG	Aminoethoxyvinylglycine
BA	Benzylaminopurine = benzyl adenine
BAP	Benzylaminopurine = benzyl adenine
BLG	Brown and Lawrence medium + gln
BSA	Bovine serum albumin
BSO	Buthionine sulfoximine
cAMP	3',5'-Cyclic adenosine monophosphate
CBM	Bornman medium
C/N	Carbon/nitrogen
D	Dark
DCR	Durzan sugar pine medium
DF	Douglas-fir
DFMA	α-difluoromethylarginine
DFMO	α-difluoromethylornithine
DCHA	Dicyclohexylammonium sulfate
DHA	Dehydroascorbic acid
dSAM	Decarboxylated SAM
DW	Dry weight
E	Embryogenic
EC or ec	Embryogenic callus
EDTA	Ethylenediaminotetraacetic acid
E_i	Embryonal initial
FAA	Free amino acid(s)
FTIR	Fourier transform infrared
FW or fr.wt.	Fresh weight
G-1-P	Glucose-1-phosphate
GA	Gibberellic acid (gibberellin)
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GD	Gresshof and Doy medium
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidized)
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HFBI	Heptafluorobutyrylimidazole
HFSE	High frequency somatic embryogenesis
HM	Hakman medium
HPLC	High performance liquid chromatography
IAA	Indoleacetic acid

IBA	Indolebutyric acid
IEF	Isoelectric focusing
IPA	Isopentenylaminopurine = 2iP
L	Larch, light or liter
LFSE	Low frequency somatic embryogenesis
LM	Litvay medium
LP	Loblolly pine
lx	Lux
MEOI	Methyleneoxindole
MES	Morpholinoethane sulfonic acid
MOI	Methyloxindole
MOPS	Morpholinopropane sulfonic acid
MGBG	Methylglyoxal bis-guanyl hydrazone
MS	Murashige and Skoog medium
NAA	Naphthalene acetic acid
NAD^+	Nicotinamide adenine dinucleotide (oxidized)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NE	Nonembryogenic
NBT	Nitrobluetetrazolium
NOAA	Naphthoxyacetic acid
NS	Norway spruce
OBHA	o-benzylhydroxylamine
ODC	Ornithine decarboxylase
P	Putrescine or phosphate
PAL	Phenylalanine ammonia lyase
pcv	Packed cell volume
PEG	Polyethylene glycol
PEM or pem	Preembryonal mass
PO	Pond pine
PP	Pitch pine
PPi	Pyrophosphate
ProA	Proanthocyanidin
RP	Red pine or research plan
S	Suspensor
SAM	S-adenosylmethionine
Sd	Spermidine
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SE or se	Somatic embryo
S_i	Suspensor initial
SIM	Selective ion monitoring
Sp	Spermine
TLC	Thin-layer chromatography
TrpAM	Tryptamine
2iP	Isopentenylaminopurine
UDP	Uridine diphosphate
UDPG	Uridine diphosphate glucose
UTP	Uridine triphosphate
WC	Wild carrot
WCM	Wild carrot medium
WH	White's medium
WP	White pine
WS	White spruce

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